Community Based Strategies to Enhance Coral Reef Resilience and Recovery through Selective Coral Larval Culturing to Strengthen Population Genetic Fitness

Chad Scott

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Author: Mr. Chad Scott

Major Program: Masters In Ecology (International Program)

Major Advisor

.....

(Assoc. Prof. Dr. James D. True)

Co-Advisor

.....

(Assoc. Prof. Dr.....)

Examining Committee

.....Chairperson

(Assoc. Prof. Dr.....)

.....

(Assoc. Prof. Dr.....)

.....

(Assoc. Prof. Dr.....)

.....

(Dr.....)

The Graduate School, Prince of Songkla Univeristy, has approved this thesis as partial fulfillment of the requirements for the Master of Science Degree in Ecology (International Program).

.....

(Assoc. Prof. Dr.....)

Dean of Graduate School

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Abstract

Coral reefs are one of the most environmentally and economically valuable ecosystems for tropical nations, but also one of the most threatened. Their narrow habitat range and reproductive strategies make them especially susceptible to anthropogenic threats and climate change. In 2010, reefs throughout the South China Sea experienced mass coral bleaching and mortality, which was particularly bad for Thailand's reefs, with up to 98 % bleaching and 90% mortality in some areas, and being amongst the structurally and functionally important *Acroporidae, Pocilliporidae,* and *Favidae* corals. Due to the frequency of these large disturbances, combined with other chronic threats, many reef areas have become so depleted that it is unlikely they can recover on their own. Both passive and active restoration of depleted reef ecosystems is necessary so that related economic and ecological values are not lost. To be effective, coral restoration programs must make a transition from the traditional methods focusing on increasing coral abundance on reefs (using cloning or other methods that reduce genetic variability of populations) to strategies focused on increasing the genetic diversity of restored reefs. Methods for the culturing of coral larvae are well developed within the scientific community, but to date most of the work being done has focused on the culturing of coral larvae for scientific research, and not for restoration. Although studies have been completed on the need the role of genetic variability and hybridization in the adaptation of corals to changing conditions, no practical guides have been written to direct local reef managers. Through the knowledge and methods gained through this study, a practical guide to integrating theories of increasing the genetic variability of feedstocks and hybridizing corals has been written. The guide provides an argument for genetic based management systems and practical procedure for carrying out the selective coral breeding and culturing project using volunteer teams and locally available materials. Such guidelines are strongly needed to preserve or restore the resilience of coral reefs in the face of a rising consortium of localized and global threats associated with human population growth and climate change currently being experienced by reefs across the globe.

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List of Abbreviations and Symbols

CCA	Crustose Coralline Algae
DC	Dead Coral
DMCR	Thai Department of Marine and Coastal Resources
EMP	Koh Tao Ecological Monitoring Program
H	Healthy Coral
NHRCP	New Heaven Reef Conservation Program
PBL	Partially Bleached Coral
PSU	Prince of Songkla University, Hat Yai
RKC	Recently Killed Coral
SEA	South East Asia
SKT	Save Koh Tao Community Group
SST	Sea Surface Temperature
USD	United States Dollars

Chapter 1: Current status of Coral Reefs and Reef Management

1.1 Introduction

Coral reefs are one of the earth's most valuable, but also one of the most threatened ecosystems. Their narrow habitat range, long generation times, and reproductive strategies make them especially susceptible to chronic anthropogenic threats and climate change. Coral reef bleaching is to becoming more frequent and severe in the last 30 years, decreasing reef abundance and diversity when combined with other stresses (Brown 1997, Hoegh-Guldberg 1999, Walther *et al.* 2002). In 2010, reefs throughout the South China Sea experienced mass bleaching and mortality due to increased sea surface temperatures associated with a strong El Nino cycle. The year was particularly devastating for Thailand's reefs, with up to 100% bleaching and 70%-90% mortality in many areas, mostly amongst the structurally and functionally important *Acroporidae* and *Pocilliporidae* corals Chavanich 2012) This event prompted the closing of many popular dive sites in the Andaman Sea, greatly affecting the Thai tourism industry. Due to the frequency of these large disturbances, combined with other chronic threats, many reef areas have become so depleted that it is unlikely they can recover on their own, or will take such significant amounts of time that their dependant economies will be severely impacted (Wilkinson 2008, Rinkevich 2008, Bruno and Selig 2007). In many cases, passive and active restoration of depleted reef ecosystems is necessary so that the related economic and ecological values are not lost.

Active coral restoration has for decades received mixed levels of support or condemnation from the scientific community. Much of the criticism for coral restoration involves a focus on solving the symptoms without addressing the root problems, or being of too small scale or scope. Other criticisms lie in the focus on fast growing and easily propagated species at the expense of coral diversity on the restored reefs (Edwards and Clark 1998). In order to be effective, coral restoration programs must make a transition from the traditional methods of focusing on increasing coral abundance on reefs (using cloning or other methods that reduce genetic variability of populations) to genetic based active restoration. Although asexual propagation techniques are well described and accepted (Edwards 2010, Yeemin *et al.* 2006), current data shows that their benefits are primarily short-term, and long term effects are generally neutral or negative (Shearer *et al* 2009, Precht *et al.* 2005). Due to these factors, reef managers should focus on increasing long-term resilience and assisting in the adaptation of structurally or functionally important corals to changes in climate or water quality.

Recent innovations and new techniques involving the culturing of coral larvae are available that can allow reef managers to imitate natural process to increase genetic variability within restored reefs. Benefits of these methods include reducing generation times, increasing reproductive success, increasing genetic or genotypic variability and reducing genetic 'bottle-necking'. All of which are important factors in the long-term survival of reefs facing a consortium of local and global threats.

This project aims to assist community level programs, which rely most on the reef health, to create selective coral breeding programs that produce genetically diverse feedstocks for active restoration. Although this technique is relatively new to coral reefs, it has been known for thousands of years that by selectively breeding plants and vertebrates, desirable or higher fitness genes can be artificially assisted to improve population tolerance to marginal conditions (Willis *et al.* 1997, Baums 2008). Long term increases in genetic fitness not only benefits the reef, but may be more cost effective than methods focused on asexual propagation, and will thus be more attractive to local reef managers.

Through this study, coral colony resilience was observed during and subsequent to the large scale mass bleaching event of 2010 on the island of Koh Tao. The local timing of coral spawning was determined using histological and field observations to then use the published methods of coral gamete collection and rearing to crossbred selected coral colonies. The resulting cultured larvae were then transferred to in-situ coral nurseries adjacent to restoration zones and tracked over the course of the study. The process was repeated over three years, to improve the techniques and develop more cost effective and efficient materials and methods to suit the community based reef management model of the island.

This study was expected to take place over the course of two years, building on the pilot work already done in 2010 alongside Dr. James True of the Prince of Songkla University, Hat Yai. Ecological and histological surveys were conducted throughout the entire study period, the mass coral bleaching event of summer 2010 meant that the corals did not spawn in 2011, and the spawning or larval culturing studies were unable to be implemented. The spawning event of 2012 was used to test and refine the basic procedure and materials from the culturing techniques utilized in 2010, and also train local reef managers in the procedure. The study was then extended to include 2013, in which the techniques published herein were compiled into a manual and a volunteer group of divers was used to carry out the procedure according to the selective coral breeding hypothesis of this paper.

This study took place over a period of four years, but the long term effects of the study will be monitored well into the future by local partners at the study site. The techniques utilized or developed during the coral culturing process developed through this study carried

out on Koh Tao have been compiled into a restoration guide for local reef managers. The aim of this guide will be to first highlight the importance of increasing or maintaining high genetic variability in restoration projects, and secondly to outline a simple procedure for the process which utilizes low cost and locally available materials and methods. The resulting manual will be printed and distributed or sold to local and community based reef managers in SE Asia.

1.2 Current Status of Coral Reefs and Reef Management

1.21 Diversity and importance of reefs

Coral reefs are the earth's most phylogentically diverse ecosystems, supporting between 1 and 9 million species from 32 of the 33 extant animal phyla (Sale 1999), and also one of the most productive and vital (West and Salm 2003). Coral reefs account for less than 0.2% of the ocean floor, but are home to about 25% of all sea life, including sponges, worms, mollusks, crustaceans, reptiles, and fish (Castro and Huber 2007.) Furthermore, they are important in protecting coastal areas from erosion and regulating atmospheric gases and global nutrients (Wilkinson 2008).

Studies have found that about 100 million people depend on coral reefs for their livelihoods (Koop *et al.* 2001). Coral reefs have historically provided food, raw materials, medicines, and other services to substance economies around the world, and more recently have contributed greatly to national GDPs through reef tourism. In 1992, the world tourism market traded $1.9 Trillion USD, while the fishing industry traded about $27 Billion USD (Birkeland 1997). In other studies, the overall contribution of reefs to world economies is estimated at 375 Billion USD per year (Costanza 1997, West & Sale 2003). In Thailand, many islands and villages are completely dependent on their reef tourism to support their economy. Specifically to Koh Tao, out of the 300,000-400,000 visitors per year, 60% are involved in SCUBA diving, and 90% partake in snorkeling activities (Save Koh Tao , unpublished,, 2008).

1.22 Threats and Status

There is an extensive amount of research showing that global coral reefs are highly threatened or degraded. Numerous studies have shown through climate modeling and trajectory patterns, based on historical and current data, that the world's coral reefs may be close to extinction within the next few decades (Costanza 1997, Wilkinson 1999, Hoegh-Goldberg 1999, Pandolfi *et al.* 2003; West and Sale 2003; Goreau 2005). Coral reefs are poorly protected, highly

degraded, and exposed to many ongoing threats. It is estimated that between 40-60% of the worlds reefs will decline over the next 50 years, and that 80% of the reefs in South East Asia are at risk (Risk 1999, Bruno & Selig 2007, Rinkevich 2008, Wilkinson 2010). In the *Status of the Coral Reefs of the World: 2008* (Wilkinson 2008) it was found that about 60% of the population of South East Asia (SEA) live within 60 km of the coast, making reefs in SEA some of the most valuable in the world, at about $12.7 billion USD . Yet, it was found that by 2008, about 20-40% of reefs in South East and East Asia were already destroyed, with another 20-22% at a 'critical' threat level. The major direct stresses and disturbances to coral reefs are well documented and include; pollution, sedimentation, nutrification, structural damage, over-extraction or use, and habitat destruction. Specifically to Thailand, the report listed over-fishing and destructive fishing as the most salient localized threats to reef health.

The most important and salient threats to global reef health today is mortality due to bleaching and diseases brought on by climate change and localized declines in water quality (Wilkinson 2008). There have been 7 major bleaching events in the last 30 years, the worst of which was 1998 when 16% of the world's hard corals died (Huegh-Goldberg 1999). Community structure of reefs has been substantially altered due to thermal bleaching events, with almost complete expiration of heat intolerant corals in some cases. The worst hit corals, both globally and in Thailand, tend to be the fast growing genera of *Acropora* and *Pocilipora* (Brown 1997, Hoegh-Guldberg 1999, Walther *et. al* 2002, Spencer 2000, Loya 2004, Ministry of the Environment 2010, Chavanich *et al.* 2012). Tropical ocean temperatures have increased by 1° C in the last century, and ENSO events have increased in intensity and frequency; and this trend is expected to increase rapidly in the next 50 years (Reviewed by Walther *et. al.* 2002, Hoegh-Guldberg 1999). Furthermore, data currently being analyzed about the global bleaching event of 2010 is showing that these predictions are materializing faster than originally predicted.

The year 2010 is tied with 2005 for the hottest year on record in the 20th century, and global ocean temperatures in 2010 were tied with 2005 for the third hottest year on record (NOAA 2011). Temperature induced coral bleaching in 2010 was particularly severe in Thailand, roughly 80% of corals bleached in Gulf and Andaman Sea bleached, with 5-40% mortality by May (Chavanich *et al.* 2012, Yeemin *et al.* 2012, PMBC 2010). On the Island of Koh Tao, in the Gulf of Thailand, up to 98% bleaching was found by May 20th, with mortality rates of 45% by late July and 78% by October (personally collected data). Even the relatively resilient population of *Fungidae* corals experienced mass bleaching during this time (Hoeskema *et al.* 2012). Subsequent to the bleaching, further mortality was observed due to algal overgrowth and disease (unpublished personal data) and predation (Hoeskema *et. al* 2013). Coral mortality can last for months after a large disturbance, as coral bleaching greatly reduces the ability of an ecosystem to withstand other threats and stresses.

Corals are relatively primitive organisms whose life cycles and morphology are greatly controlled by environmental conditions. Populations made up of long-lived organisms such as corals are slow to adapt to changing conditions. Chronic and severe disturbances such as the worldwide mass bleaching events of 1998 and 2010 are outside of the natural evolutionary structuring of reefs, and can lead to algal or sand/rubble dominated ecosystem shifts (Edwards and Gomez 2007). Due to their narrow ecological and geographic range, corals will be more affected by climate change than most other marine species. Generally, species may track and change with the changing climate towards the poles, but in corals this will not occur due to limitations imposed by temperature and light levels (Reviewed by Walther *et al.* 2002). Recent publications have raised theories that corals will adapt to climate change (Baker 2001, West and Salm 2003, Baker 2008, Guest *et al.* 2012). Yet many researchers disagree with the theories (Riegl *et al* 2011, Bongaerts *et al* 2010), and additionally, these theories generally do not take into account the consortium of effects related to climate change; instead focusing on a single variable or specific threat in isolation.

There is currently no scientific consensus on the future projections for coral reefs, or on the degree of resilience and adaptation they will exhibit (Baums 2008, Knowlton 2001). While researchers continue to debate the future projections for coral reefs, the fact that they are quickly being depleted cannot be ignored. It is unlikely that corals will adapt to climate change and rebound in any reasonable human time scale. On Koh Tao in 2010, about 68% of the *Acropora*s died in Chalok Ban Kao, implying that possibly the other 40% had some physical or genetic reason for surviving. Yet, subsequent to the bleaching, many of the surviving corals perished due to disease, overgrowth by algae, and predation by what was then an overpopulation of *Drupella* snails (Hoeskema *et al.* 2013). Even if coral can adapt slowly to one threat, there are many more stresses and chronic disturbances related to anthropogenic activities affecting them, it is highly unlikely they can adapt in a timely manner to them all.

1.23 Reef Management in the Face of Climate Change

The effects and mechanisms of coral bleaching and related coral diseases are well documented, and will not be reiterated here. But, it should be noted that there has been "an unacceptably long delay" between the identification of the problems affecting coral reefs and the establishment of the necessary monitoring, protection, and restoration programs to address the problems (Risk 1999). Most management techniques implemented over the last 20 years have been focused around designation of Marine Protected Areas (MPAs), and other passive management techniques. Despite all management efforts implemented to date, reef decline has risen from 1% per year before 1997, to 2% between 1997 and 2003 (Bruno & Selig 2007, Rinkevich 2008,). By 2008, it was estimated that the good and services of 19% of the world's

coral reefs have been completely lost (Wilkinson 2008). The results show that protective measures are not enough, and that restoration and disturbance mitigation projects are vital for the survival of coral reef ecosystems and related economies (Edwards & Gomez 2007, Edwards 2010). But, necessary restoration has been delayed because much of the funding and time spent on coral reef research is still focused on the idea of understanding problems and mechanisms, even repeating much of the work already completed throughout the last century.

Many studies cite that the broad and indirect ecological or economic effects of decreased coral diversity on reefs remains "essentially un-investigated"; our knowledge both of climate change and coral ecosystem responses are inadequate (Knowlton 2001). The continuation of this belief leads to an inappropriate amount of funding going towards research instead of restoration. Other papers also cite the lack of information about the past or current state of world reefs (the so called 'shifting baselines syndrome') as a barrier to restoring reefs as mangers cannot possibly return them to their previous state without knowing exactly what that state was (Pauly 1995, Knowlton 2008). But, as quoted from Sale (1999) "because coral reefs are dynamic, living systems, management should focus on the dynamics rather than the state." Despite the lack of knowledge on reef ecosystems and baselines, action is needed to mitigate further losses and restore the function and value of reefs in areas that have been lost. Instead of focusing resources on restoring species that have been unable to survive changes in the local reef system, resources should be allocated to restoring functionally or biologically similar species that can provide the same ecological services as those they replace.

This unacceptably long delay in active restoration needs to be addressed, reef scientists need to heed the precautionary principle "Where there are threats of serious or irreversible damage, lack of full scientific certainty shall not be used as a reason for postponing cost-effective measure to prevent environmental degradation" (Risk 1999). In the face of chronic anthropogenic threats and increasing climate fluctuations, passive and active coral restoration is critical for the maintenance of reef ecosystems and dependant economies (Edwards and Gomez 2007, Baums 2008). Following mass disturbance events, recovery of the ecosystem requires the settlement of new coral larvae originating from within the local reefs or from remote, yet connected, reef areas. Reef connectivity is a popular field of inquiry worldwide as scientists race to understand the recovery potential of fish stocks and corals.

Coral reefs are becoming more fragmented and isolated as the abundance and density of reproductively viable adult colonies decreases. Currently, the potential for flow of larvae between coral reefs separated by 100's or 1,000s of kilometers is not well understood for most coral species (Sale 1999). As further ecosystem fragmentation is experienced, and reproductively viable adult colonies become sparser and more isolated, passive protection measures will not be enough to sustain the flow of larvae needed to replace the adult colonies being lost. As reefs

decline, MPA's will become even less effective as the sole method of management, and restoration programs are required to maintain reef biodiversity (Hoegh-Goldberg 1999; Sale 1999; Wilkinson 1999; Knowlton 2001; Pandolfi *et al.* 2003; Goreau 2005; True 2009). Without active management and restoration, coral reefs may lose the ability to recover even if anthropogenic threats are decreased or halted. Although necessary, coral restoration is still a developing industry which has yet to catch up with current scientific knowledge. The development of more scientifically directed restoration programs may one day increase the ability of corals to adapt to changing conditions and survive better in the face of climate change and rapidly increasing human populations.

1.24 Limits of non-genetic based restoration and importance of coral diversity

Evolutionary adaptation to changing conditions is the method through which organisms and ecosystems have evolved and survived. Through natural selection, changes in allele frequencies within populations increases genetic diversity which is selected for or against, leading to populations more suited for their environment or the eventual emergence of new species. In a stable-state ecosystem, adaptation occurs slowly as most mutations are deleterious to the individual, but, in changing environments this effect is reversed and a higher proportion of total mutations prove to be beneficial. High genetic diversity and short generation times would assist corals in adapting to declining water quality or climate change. Although corals may become locally adapted, little is known on the rate or degree of adaptation in corals (Baum 2008). However, if effective population size is reduced, or genetic diversity is limited within populations bottlenecking and founder effects can lead to extirpation of species from the ecosystem during large scale disturbance events.

Most mainstream coral reef restoration projects (especially those available to community based programs) have been focused on increasing reef structure through artificial reefs, increasing coral abundance through asexual propagation (cloning), or transplanting of corals through various nursery and gardening techniques (Yeemin *et al.* 2006, Putchim *et al.* 2008, Baums 2008, Edwards 2010, Shaish *et al.* 2010). Coral colonies, especially *Acroporidae* and *Poccilioporidae*, fragment and produce ramets that can increase the spatial coverage and preserve the genotypes of the parent colony (Baums 2008). Techniques for the cloning of corals through asexual propagation have been well described for many coral species around the globe, primarily in the context of aquarium corals. A thorough review by C.J Delbeek (2001) describes various techniques for propagating corals and details the locations around the globe with commercial production of coral for the aquarium trade. These techniques are thought to have first developed in the 1950's in New Caledonia, with the fragmentation of A*cropora* branching corals. Coral species or genotypes with a high stress tolerance tend to survive better in the

aquariums, and are thus propagated and sold around the world. The high success and ease of creating new coral colonies through these methods has led to profitable coral farming industries rising up and reducing the trade of corals taken directly from natural reefs.

The use of propagation in the trade of corals not only reduces the pressure on natural reefs, but can provide and environmentally sustainable livelihood to locals and allow sellers to clone and distribute high fitness corals that survive well in aquaria (Delbeek 2001). Mainstream coral restoration has in many cases copied these techniques for use in the natural environment, assuming that the same benefits will be achieved. Using propagation and transplantation of corals was first described in the mid-1970's, and by the mid-1980s had been utilized in many locations around the globe including the Philippines, Guam, Singapore, Hawaii, Florida, and The Great Barrier Reef (Clark & Edwards 1995). While many of these projects reported high short-term success, few of them carried out long-term monitoring until the study by Clark & Edwards in 1995. Their study achieved 75% survivorship after 1 year, which decreased to 51% after 28 months. Their final conclusion was that, in their specific research site, the long-term benefits (5-10 year) were small, and did not balance with the cost of removal of coral from the 'donor' areas. That "transplanted areas will only be distinguishable from unstransplanted ones by the greater amount of dead coral in the former." They did however review other studies which had greater success or potentially higher long-term environmental benefits. Since that time, the promotion and use of fragmentation and propagation in coral restoration has only increased (for example Edwards 2010, Edwards & Gomez 2007, or Fujiwara & Omori 2004). Although a sexual cloning may be beneficial in preserving successful genotypes (as in aquaria), the long-term effects on the ability of reefs to locally adapt to changing conditions may be hindered.

The length of monitoring for coral restoration projects is generally only short term, usually covering the first year (Putchim *et al* 2008, Shaish *et al.* 2008). Of the 5 case studies presented in the book *Reef Restoration Concepts and Guidelines* (Edwards and Gomez 2007), only two had monitoring programs extending longer than 1 year, with the longest monitoring program planned for a maximum of 5 years. Meaning that most researchers and coral restorationists are looking primarily only at the small scale or short-term benefits of their programs without considering long-term consequences of reduced genetic variation.

Due to the lack of long-term monitoring of coral restoration projects, asexual propagation of corals continues to be one of the most popular methods used by reef managers. In a consensus of the oral presentations related to restoration given at the 2nd Asia Pacific Coral Reef Symposium; 8 described projects using fragments collected from donor colonies, 6 described adding reef structure, only 1 focused on larvae culturing, and none focused on the utilization of naturally formed coral fragments. Of the poster presentations on coral nurseries or transplants; 3 used asexual cloning from donor corals, 3 larvae culturing, 2 natural fragments, and 1 used

fragments from an unlisted source. The two largest projects were from Bali Island, Indonesia (Onaka *et al.*) and the Gulf of Thailand (Sritongkam). The first project created 110,000 ramets of *Acropora* sp. from an unlisted number of donor colonies and monitoring was carried out for only 1.5 years. The second project has a goal of 80,000 ramets from branching *Acropora* over 5 years and did not list the number of donor colonies nor the length of monitoring.

The well described techniques of coral propagation are popular and widely used in restoration as they are generally inexpensive and information is widely accessible to local managers through freely available on-line books such as the *Reef Restoration Guide* (Edwards & Gomez 2007) and the *Reef Rehabilitation Manual* (Edwards 2010). Furthermore, the projects do not require highly skilled labor and are thus attractive to conservation groups utilizing public participation or volunteers (Scott and Phillips 2010). Short-term or small scale success has regularly been achieved by such programs in increasing coral abundance or stakeholder participation; and the dominate argument against such propagating or coral gardening techniques is scale and cost issues (Rinkevich 2008, Edwards & Gomez 2007). Almost none of these studies assessed the negative effects on 'donor' colonies or the of decreased genetic diversity due to coral cloning. The first thorough investigation of the genetic implications for coral restoration was not written until 2008 in *A Restoration Genetics Guide for Coral Conservation* (Baums). By not taking into account the reduced genetic diversity of these gardened reefs, managers will most likely only improve conditions on the short term, while almost certainty decreasing the long term resilience and adaptability of the reefs (Baums 2008, True 2009).

There have been few studies on the effects of decreased genetic diversity of reefs due to coral propagation (Baums 2008, Shearer *et al.* 2009), but terrestrial examples show that the resilience of ecosystems to pests, diseases, and other disturbances is reduced due to the effects of monoculture, cloning, and age uniformity. Compounding the problem of coral's normal slow adaption to changing environmental conditions, reproductive success in restored coral populations will be dramatically reduced as asynchronous broadcasting corals generally cannot self-fertilize. Long-term fertilization and recruitment failures can occur in gardened reefs as mono-specific stands are essentially reproductively sterile (Baums 2008, Carlon 1999 in Knowlton 2001). Additionally, barriers caused by low genetic diversity or other factors such as environmental controls, temporal or spatial isolation, low population density, and low fecundity can also lead to reproductive failure and thus the inability of reefs to adapt or rebound after large scale disturbances (Knowlton 2001).

In order for coral species to survive the next few centuries, they will need to adapt to changing climates and habitat conditions through evolutionary mechanisms of mutation and hybridization. Such mechanisms arise through attrition and reproductive success only achievable in the presence of high genetic diversity amongst populations. This can be addressed by taking a

genetic approach to coral restoration and implementing projects involving the culturing of genetically distinct coral larvae to support natural reef adaptation mechanisms (Peterson & Tollrian 2001, Baums 2008). Current management programs often overlook the importance of genetic or genotypic diversity on the basis of increasing abundance, or focus primarily on reinventing or reinvestigating methods already in use elsewhere (see Sritongkam 2010, Ng & Chou 2010, Yucharoen *et al*. 2010, Putchumin *et al.* 2008).

Even today, new publications such as that by Latypov *et al.* (2013) are being released redescribing the techniques of coral cultivation through asexual fragmentation, supporting claims of short-term success while overlooking the importance of genotypic diversity. Compounding the problem is the fact that environmental management and restoration that should be advised by scientific professionals has often been based on non-scientific motives or politics, leading to misguided or unsustainable project implementation. Often only the most easily propagated, or fastest growing corals have been used in restoration projects in order to show high success, without considering the functional or structural importance of the species being used (Edwards & Clark 1998).

Coral restoration is a necessary management tool to preserve the ecological and economic functions of coral reefs, but without taking a more scientific and long-term approach the success of such management will be limited to small scale projects addressing infrequent or intermittent events (boat groundings, anchor dropping, fish bombing, etc). Reef managers should focus on the spawning and larval life stages of corals in order to address the survival or adaptation of reefs to the chromic stresses related to climate change and increasing human populations. In order to increase the effectiveness of reef managers, the scientific community needs to create guidelines and protocols for coral restoration which consider genetic diversity yet utilize simple techniques and widely available materials.

1.25 Coral Spawning mechanisms and spawning study

Corals exhibit a wide range of reproductive methods, and are divided into two main groups: brooders and broadcasters. Brooders are hermaphroditic or gonochoric corals which internally fertilize eggs by absorbing sperm released by neighboring colonies. After development, a free swimming larvae is released into the water column. These larvae usually contain symbiotic zooxanthallae inherited by the parent colony, and are settlement competent. Dispersal ranges thus vary greatly as the larvae may settle within several meters of the parent colony or alternatively survive in the water column for up to 100 days and be transported over 100 km (Richmond 1987). Broadcasting corals are likewise hermaphroditic or gonochoric, but will asynchronously release high lipid sperm and egg bundles into the water column which float

to the surface and fertilize through direct contact in slicks formed on the ocean surface. Fertilized eggs then develop into planktonic and free-swimming larvae that can survive in the water column for as much as 195 to 244 days (Graham *et al.* 2008). Brooding corals tend to have a lower dispersal range than broadcasters, but may be more evolutionarily advanced (Baird *et al* 2009) and better suited to deal with climate change (Ohki *et al* 2013). Thus, there is more potential need for reef managers to initially develop larvae culturing programs for broadcasting corals rather than for brooders (Glynn & Colley 2008).

One of the leading knowledge barriers to using coral culturing as a method to produce genetically diverse restoration feedstock's is the lack of knowledge on, and difficulty in the collection of coral gametes. Although sexual reproduction is a critical aspect for the survival and succession of corals, there is a lack of knowledge on the factors that control reproductive events for the majority of coral species (Guest *et. al.* 2008). The majority of mass spawning broadcast corals will release egg and sperm bundles for less than 30 minutes, one time per year. In order to collect coral gametes for restoration, reef managers must time their work to coincide with this event, to be 'in the right place at the right time'. In some popular research locations spawning timing studies have been on-going for decades, such as the exceptional study of 105 coral species in the Great Barrier Reef published in 1986 by R.C. Babcok *et al*. For most remote locations around the globe, the spawning patterns of most sceleractian corals is still unknown or unrecorded (Kongjandtre *et al.* 2010, Guest 2009). One of the first obstacles local reef managers must overcome then is to identify the spawning patterns for their area and develop accurate timetables for the species which are in need of assistance through projects addressing the early life stages of corals.

Coral reefs which are in most need of larvae culturing programs are those which have limited natural recruitment success. Coral reefs with high recruitment will recover faster after a disturbance (all other factors being the same) than reefs which are recruitment limited. Therefore it is important for local reef mangers to assess the levels of recruitment on their reefs, and identify sources of larvae from connected reef systems. Currently this is difficult due to the lack of knowledge on the connectivity of reef sites in relation to distribution and larvae sources or sinks. For example, observed variations in the abundance of adults does not correlate to variation in recruitment, meaning that many factors are at play which marine scientists have yet to understand or include in most models (Sale 1999). Thailand, and indeed all of SE Asia, has been regarded as one of the areas where the least amount of knowledge or data on these factors exists (Guest *et al.* 2005, Plathong & Plathong 2006, Kongjandtre *et al.* 2010).

To date, very few reports (and primarily in Thai language) have been published on the spawning seasons of scleractinian corals in Thailand. Areas where information is available include; Phuket (Chanmenthakul 2001, Kongjandtre *et al.* 2010), *Acropora* in the Gulf of

Thailand (Piromvaragorn *et al.* 2006 in Kongjandtre et al. 2010), species of *Favia* in the Gulf of Thailand (Kongjandtre *et al.* 2010), and larval dispersal in Chonburi (Putchim *et al.* 2006). At the time of writing, there has been no spawning data published on the economically important Western Gulf region of Koh Tao or the other 'Paradise Islands.'

1.26 Reef Management for the 21st Century

Despite the lack of scientific consensus on the mechanisms and projections of coral reef decline due to pollution, diseases, and climate change; it is well known that ecosystems experience multiple stable states that will change in response to their ability to withstand and recover from disturbances and adapt to different environmental conditions (West & Salm 2003; Folke *et al.* 2004). Coral reef ecosystems should be looked at as constantly evolving systems that will adapt faster with higher genetic diversity, instead of focusing much of restoration efforts on returning reefs to historic states (when known) or temporarily increasing the amount of coral (Sale 1999, Baums 2008).

In contrast to their symbiotic bacteria and zooxanthallae, corals are long lived and thus slow to adapt. As with other organisms, their rate of adaptation will be a factor of the amount of genetic material available in the reproductively successful population and the lag time between generations. In addition to genetic mutations, simultaneous spawning of corals provides the potential for hybridization, and *ex situ* tests have confirmed that this can occur even between species with different morphologies (Hatta 1999, Vollmer & Palumbl 2002, Willis *et al.* 2006). Closely related species generally spawn within a close time span, which increases the potential for hybridization (Willis *et al.* 1997, Kongjandtre *et al.* 2009). The focus of coral reef management should be on decreasing generation times and increasing the genetic diversity available within reef populations to mitigate the loss of ecosystem services and dependant economies and facilitate faster adaptation to changing environments (Baums 2008). The only restoration methods which allow for increasing genetic diversity of corals on a realistic scale are those that increase the success of sexual reproduction in corals through the culturing of coral larvae.

1.27 Rearing of coral larvae to improve diversity

To mitigate the lost of ecosystem services and dependant economies, coral reef management should focus on increasing the genetic diversity available within reef populations and attempt to facilitate faster adaptation to changing environments. Currently there is little information available offering guidance to local reef managers and, although techniques have been described for the use of sexually produced corals in restoration, so far they have been more

expensive and require greater training than methods directed at propagation of corals through asexual means (Peterson & Tollrian 2001, Heyward *et al.* 2002, Edwards 2010, Omori *et al.* 2010). The techniques and science are not yet proven consistently, and before the techniques will be available to local reef managers it is necessary to find sustainable and cost effective methods of coral culturing.

Only a limited number of species have been successfully studied and reared through larvae culturing, mostly from *Acroporidae* and *Faviidae* (Guest *et al.* 2010). These techniques have been carried out for this limited number of species mostly amongst researchers using specialized equipment and techniques (Peterson 2001, Heyward 2002, Edwards 2010). In Thailand, there is only one other program focusing on using corals cultured from larvae in restoration projects (Chavanich, personal communication). There is very little information available offering guidance to reef managers and non-scientists about how to rear and maintain coral larvae for use in reef rehabilitation and disturbance mitigation. The techniques and materials required are usually beyond that of the capacity for many local reef managers, and it is necessary to develop methods utilizing locally available materials and adaptive techniques for non-scientists.

1.28 Selective larval culturing for restoration

Current trends demonstrate that the policies and individual actions necessary to effectively decrease the stresses and mass mortality of corals caused by human activities and global climate change will not be implemented in time. Climate change, alteration of sea water chemistry, and habitat destruction is happening at a pace to fast to stop. Efforts should be focused on increasing the genetic diversity of the corals reefs in order to speed up the natural selection and evolutionary processes that have allowed corals to survive ice ages and other global disasters. Given time, Coral will adapt and repopulate disturbed areas, but this could take hundreds or thousands of years, thus eliminating the services reefs provide to the global environment and economies. This natural process of succession can be artificially altered through developing strategies aimed at increasing the success rate of larvae recruitment on reefs. This can be done through the collection of coral gametes which are raised *ex situ* and then released back onto the natural reefs.

There are many inherent benefits to using coral gametes to provide restoration feedstocks as opposed to coral propagation from ‘donor’ colonies or use of naturally formed coral fragments. One of the most significant benefits is the fact that each coral colony produced through larvae culturing is a genetic individual. As genetic individuals, proportionally they have a higher overall chance of long-term survival and reproduction in the face of variable conditions

then ramets created through cloning. As opposed to the mono-specific or low diversity stands created through propagation, restored reefs utilizing larvae culturing methods will have a higher reproductive potential through reduced founder effects or genetic bottlenecking, less reproductive failure due to self-fertilization barriers, and decreased generation times through higher larval survival rate. Furthermore, long-term hybridization or selective breeding of coral potentially could lead to more heat or stress tolerant corals that could better withstand the consortium of threats associated with climate change.

Hybridization has most likely played an important role in the evolutionary history of corals (Hatta *et al.* 1999, Vollmer 2002), and intraspecific hybrids may show fitness advantages compared with their parents when grown in marginalized environments (Willis *et al*. 2006, Baums 2008). Transfer of genetic mutations within corals and other gene exchanges between populations or species through cross breeding is likely to be a major factor in the ability of corals to respond to climate change (Van Oppen *et al.* 2003). A review by Willis *et al.* (2006) argues that the reticulate evolutionary history of corals suggests that hybridization has played a major role in the diversification of coral genera such as *Acropora*. And throughout the evolutionary history of corals, hybridization events have been an "important source of raw material for radical evolutionary change." Furthermore, the authors concluded that hybrid corals effectively colonize marginalized habitats in which the parent species are unable to survive and increases the "range expansion and adaptation to changing environments." Meaning that in areas where coral abundance is declining, hybrid species may be able to invade and replace the structural or functional diversity being lost. Most importantly to this study, the authors concluded that

> Hybridization [is] likely to be significant for the future resilience of reef corals, for example, by providing options for rapid response to changing environments and climatologies as well as increasing resilience to novel disease challenges. Hybridization warrants consideration when developing conservation strategies for corals.

The study did not, however, explain how current coral conservation or restoration methods could utilize hybridization, nor did the study offer examples of novel restoration methods.

Corals show a high propensity for hybridization relative to other invertebrates, and it was recently discovered that what was long thought to be a distinct species of staghorn coral (*Acropora prolifera*) in the Caribbean is actual a hybrid of two other species (Van Oppen *et al*. 2003). Broadcasting corals such as those from the genus *Acropora* tend to spawn simultaneously, leading to a high contact of gametes by different species (Van Oppen *et al*. 2003, Willis *et al.* 2006). Hybridization and selective breeding for desired traits has been well established in the agriculture and animal farming to increase growth, yields, or stress tolerances. It is not well known however which coral genera may have similar propensities for hybridization, and further

work is needed to determine the possibility of hybridization between particular species or genera of corals (Kongjandtre *et al*. 2009).

Several studies have been undertaken in the experimental hybridization of scleractinian corals to identify breeding compatibility between species (Willis *et al.* 1997) or in using molecular tools to identify rapidly evolving genes (Aranda *et al.* 2012). To date, no literature is available providing guidelines for the use of hybridization in coral restoration. By coupling the information on the necessity for high genetic variation and role of hybridization in adaptation with coral larvae rearing techniques, it may be possible to positively influence the adaptation of corals to changing climates and declining water quality being experienced around the globe.

1.3 Study Site: Koh Tao, Thailand

Koh Tao is a 19 km^2 island located in the Samui archipelago in the Western Gulf of Thailand. The island is relatively remote and distant from the main land, located 72 kilometers from Chumphon City to the west and 115 kilometers from Suratthani city to the South (see Map in Appendix A). The island is surrounded by dense fringing reefs and large coral heads or granite pinnacles surrounded by sand. Data collected through the local Ecological Monitoring Program (Scott 2012) shows the *Acropora* as the dominate growth form of coral around the island, followed by *Porites*, *Pavona*, and *Montipora* (23%, 14%, 12%, and 10% of total coral coverage, respectively). Preliminary results of a 2013 study show a marked difference in the composition of corals between 'high' use and 'low' use sites around the island. 'Low' use sites tend to be dominated by Branching corals (mostly *Acropora)* and have a low abundance of Foliose (mostly *Pavona)* growth forms (mean abundance 35% and 15%, respectively). 'High' use sites tend to have very litte Branching coral coverage, and are instead dominated by Foliose Corals (mean composition 10% and 30%, respectively) (Hein, in Prep.).

Koh Tao receives around 300,000 visitors per year, of which an estimated 60% are in some way related to the local diving industry. Thus SCUBA diving and reef related tourism forms the base of the local economy (Larpnun *et al.* 2011), and in 2009 the island was responsible for 46% of all the PADI diving licenses issued in Thailand (PADI International, personal communication). The island has over 45 dive businesses, and hosts approximately 3,000 dives per day in the high season, mostly at just 6 of the island's 23 dive sites (Weterings 2011, Scott 2010). A study conducted in 2012 (Nichols 2013) conducted surveys with 29 of the 46 dive schools on the island and estimated that around 299,390 divers visit the island each year. Each diver was assumed to make an average of 14 dives, meaning that some of the popular dive sites may experience over 300,000 dives per year.

Koh Tao has experienced a surge of tourism and rapid development over the last two decades. In a report by Weterings (2011), remote sensing data shows that in 1975 the island was covered by 1,527 ha of tropical forest, with only about 3.2% of the island altered by humans (mostly for coconut farming). By 1994 the amount of native forest had dropped to 1,263 ha, by 2001 it was down to 1,266 ha, and by 2005 only 967 ha of native forest remained (a total decrease of over 36% in 30 years). Many facilities on the island are underequipped to deal with the large influx of residents and visitors such as electric generation (Saengsrithorn and Kitworawut 2010), freshwater (Larpnun *et al*. 2011), waste management, and wastewater treatment. These localized threats to reef health are exacerbating regional threats such as water quality decline and over-fishing, reducing the resilience of the corals reefs to climate change or large natural disturbances.

'Top-down' governance on the island promotes unchecked growth with very little protection of the island's resources, and very few restrictions or regulations on terrestrial or marine activities have been adopted or enforced (Larpnun *et al.* 2011). Due to the lack of government initiative or strength, local stakeholders have been identified to have the most interest in protecting local ecosystems on Koh Tao, therefore, more recently, government groups have focused their efforts at increasing local stakeholder capacities (Larpnun *et al.* 2011). Members of the local community have created a community group called Save Koh Tao in order to implement necessary social and environmental projects. This group works to educate and support local business in getting involved or even creating their own programs to offset the externalities of the tourism industry (Scott and Phillips 2011).

Since 2007, the practical management of marine resources on the island has been largely left to the Save Koh Tao (SKT) Marine Branch, which consists primarily of local dive school owners and employees. These local stakeholders are directly connected to coral reef tourism to sustain their livelihoods, and are also most aware of the problems being experienced. This unique situation provides a good platform to experiment with developing techniques of coral restoration and protection available to local level reef managers. The SKT Marine Branch works closely with the Department of Marine and Coastal Resources, the Department of Fisheries, and scientific advisors from Prince of Songkla University and Mohidol University International College. Monthly community meetings are hosted to raise awareness and encourage involvement in upcoming activities, and the projects conducted each month are carried out by volunteers from the dive centers. Projects include maintaining mooring buoys, monthly clean-ups, artificial reef construction and maintenance, coral nurseries, and much more. The SKT Marine Branch also encourages dive centers to run daily environmental related courses, with 5 dive schools on the island already hosting such programs.

The first conservation based dive program on the island was the New Heaven Reef Conservation Program, started in 2007. The NHRCP runs 2 to 4 week courses, and up to 6 month internships encompassing many aspects of reef research, protection, and restoration. This program was selected to be the location for the pilot spawning and culturing project on the island has it had the largest base of student volunteers, runs 7 days a week, and has interns that are trained in the scientific and diving related skills necessary to carry out the project tasks.

1.4 Project Objectives

The broad goal of this project is to develop and provide methods and knowledge to local reef managers to enhance coral reef resilience in the face of chronic disturbances and climate change. More specifically:

- Map the loss of hard coral coverage in 2010 around the island of Koh Tao following the mass bleaching event to document the amount of hard coral lost and identify more or less resilient reef areas around the island.
- Identify coral colonies that survived the best through the 1998 and 2010 mass bleaching events, especially those in the same microhabitat where mortality rates were high, and attempt to increase the reproductive success of those populations through coral larvae culturing.
- Review the role of hybridization in the adaptation of coral reefs and attempt to provide practical methodology to local managers to utilize hybridization or selective coral breeding in restoration activities
- Implement the selective coral breeding program in marginalized areas on the island of Koh Tao to restore coral populations and community structure juveniles cultured from the most resilient and robust coral populations
- Monitor and research the effectiveness of the restoration techniques both on reef health and on the local community and economy.
- Compile information and methods into a publication that can serve as a guide to local communities and reef managers for increasing reef resilience through a focus on coral genetics.

Chapter 2 -Research Methodology

2.1 Assessing the impact of bleaching in 2010 around the island of Koh Tao

During the bleaching event of 2010, ecological survey data was collected using belt and point intercept transects along a 100 meter permanent line. Each location surveyed has 2 lines (shallow and deep), which are broken up into 4 sections to yield a total of 8 twenty meter segments per site. Data were collected according to the locally designed Save Koh Tao Ecological Monitoring Program (Scott 2012), based on methods of the CPAD Foundation and Reef Check International, but with indicator species and coral assessment modified to fit local needs (Phillips *et al.* 2010). Eight locations around the island have permanent lines, with 4 years worth of data, plus an additional 3 sites were added during the bleaching event to increase project scope.

Within each survey segment, the substrate type is identified by points falling under the line every 50 cm within 10 categories (Silt, Sand, Rubble, Rock, Sponge, Soft Coral, Hard Coral, Trash, and Other). If the substrate type is hard coral (HC), then the growth form (Branching, Corymbose, Digitate, Encrusting, Foliose, Laminar, Massive, Solitary, Submassive, and Tabulate), followed by the health (Healthy, Partially Bleached, Fully Bleached living, Recently Killed, and Dead) is recorded. Fish and invertebrate indicator species abundance and diversity was also monitored during each survey, but was not included in this report. At the onset of bleaching the methods for the Compromised Coral Health Survey as established by GEFCoral and the *Coral Disease Handbook* (Raymundo 2008) were adopted into the program. Additional data was taken on coral health, including: taxonomy to genus level, percent of colony bleached, type of bleaching (Pale, Molted, White), percent mortality (recently killed or dead), disease presence, and cause of mortality (if other than bleaching).

Additionally, in situ HOBO UA-002-64 Pendant Temp/Light probes were installed on February 5th, 2010, at varying depths and locations around the island. Locations and depths where: Chalok Ban Kao (4m and 9m depths), Hin Fai Biorock (N10'06.743", E099'49.124, 11m Depth), Ao Leuk (N10'03.985", E099'50.488", 6m and 10 m Depths), and Sai Nuan (N10 04'41.34", E99 48'58.60", 8 m depth). Probes were set to record data every 2 minutes. Light and Temperature data was plotted using HOBOware Pro Software and Microsoft Excel.

Subsequent monitoring will continue after the duration of this project to track ecosystem health and recovery and impact of future disturbances, focusing primarily on the areas found to be most or least resilient during these initial surveys.

2.12 Identifying resilient coral colonies

Data collected through the Ecological Monitoring Program was coupled with observations on bleaching and mortality. Areas found to have been close proximity or similar physical conditions, but with a large discrepancy in mortality rates, were deemed to be more or less resilient than the baseline. In the sites deemed to be more resilient, coral colonies were mapped and the size of the colonies was recorded. Three collection sites were chosen from this data that contained each of the two coral genera for which local spawning timing information was available. The three locations in Chalok Ban Kao Bay were chosen based on what were assumed to be three distinct breeding populations; populations far enough apart to have a low chance of cross fertilization is a mass spawning event. It is assumed that to a large degree each one of these populations would normally only fertilize gametes with their close neighbors and lead to founder effects or genetic bottlenecking. Artificially cross fertilizing these three populations it may be possible to increase the genetic diversity of the reared corals or allow the transfer of locally adapted genotypes.

For each site, or distinct breeding population, only the location and size of colonies assumed to be of reproductive size, greater than 25cm minimum diameter, were recorded. Site 1 consisted of 2 *Symphyllia* colonies and 8 *Goneastrea* colonies. Site 2 contained 3 *Symphillia* colonies and 18 *Goneastrea* colonies. Site 3 has to be split into two areas, one for *Goneastrea* and one for S*ymphillia*, the *Goneastrea* site (Site 3A) contained 14 colonies, while the *Symphillia* site (Site 3B) contained 5 colonies (Figure 5, in Appendix D). The distances between the sites were 76 meters, 63 meters, and 131 meters (Site1-2, 2-3A, 2-3B, respectively).

2.13 Establishing schedules of coral spawning

Local timings of coral spawning was estimated using available publications for the region for *Acroporidae* and *Favidae* corals (Kongjandtre 2010, Piromvaragorn et al. 2006). From the literature, the month of spawning for these families was predicted to be between February and April, shortly after sunset, 3-6 days after the full moon.

From January till April of the study period (2010-2013), coral were examined at regular intervals of about 2 weeks for pigmented eggs through on-site histology. This consisted of trained volunteer divers extracting polyps in the regions of the colony with the predicted highest fecundity and visually observing the presence or absence of eggs as per Wallace 1985. Pigmented egg bundles (pink or red) are likely to be released in a mass spawning event following the next full moon (Baird *et al.* 2002), and diver teams were deployed to observe the corals nightly.

For the months of peak spawning, a team of volunteer divers was recruited to continually observe selected coral colonies from 1-7 nights after the full moon (sunset to 11 pm). Divers were supplied with cameras to record any reproductive activity, aid in identification of coral species and behaviors, and also provide time stamps for the start and end of spawning for various species. A total of 6 such weeks of observations were carried out over the course of the study, as in 2011 no corals were found to produce gametes following the bleaching event of 2010.

A database of coral spawning for the species observed was created to relate the time of spawning to the lunar and circadian cycles. The spawning timings were correlated to the number of days and hours following the full moon (Greenwich Mean Time), and then projected into the future using a calendar of full moon timings. This will be used to reduce the time and resources needed to have constant observation of the corals by divers by predicting spawning timings more accurately in the future.

2.14 Constructing the culturing tanks

Coral culturing units were constructed at the New Heaven Reef Conservation Program (NHRCP), in Chalok Ban Kao. For the projects carried out in 2010, the units were made of plastic tubs or buckets, and water changes were carried out by siphoning water from the units and with volunteers walking or taking a boat into the sea to collect fresh sea water. Although water quality could generally be maintained, the project experienced prolonged periods of poor water quality as water changes were tedious and not entirely effective. In 2012 the plastic tubs were modified with stand-pipes and a pump to provide continuous water circulation and more effective water changes.

For the project in 2013, permanent culturing units were constructed using rendered cement that was flushed with sea water and vinegar continuously for 2 weeks to balance pH. The system consists of 3 coral culturing tanks (W 100cm X L 100cm X Depth 60cm) and two larger tanks for other organisms (W 100cm X L 100cm X Depth 60cm) and a final water treatment/macro-algal tank (W 100cm X L 120cm X Depth 40cm). Water flow is provided continuously by a submersible pump, and flow can be controlled independently in the individual tanks. Stand-pipe designs and gravity provide for passive flow of water from the tanks to the mechanical and biological filters for recirculation (see Figure 6 in Appendix E).

The filtration system consisted of a top layer of aquarium meshing (5-8 cm thick), a layer of activated carbon, a partially submerged layer of store bought Bioballs™, and a final layer of coarsely crushed clay brinks. Water flowing out from the filtration unit was passed first through baskets of coral rubble to balance pH and maintain calcium levels before being strongly

circulated and aerated by a venturi system diverting water from the submersible pump. A 10-20% water change was preformed daily by releasing water from the sump tank and refilling with fresh sea water pumped through the filtration unit. The units are approximately 5 meters from the beach, and 2 meters above the winter high tide.

The coral culturing unit tanks were covered with a roof made of UV blocking transparent polycarbonate plastic. Coral larvae require and use ambient light levels (Gleason and Hoffman 2011), but are negatively affected by UV light (Wellington and Fitt 2003, Gleason *et al*. 2006), the clear roof helps to maintain the visible light spectrum at near ambient levels while reducing harmful UV spectrum light. Covering the tanks is essential as rain water can reduce the salinity of the tank water, and studies have found that linear decreases in salinity yield exponential rates of coral larvae mortality (Vermieji *et al*. 2006). The tanks must also be protected from falling leaves and organic matter, as corals naturally occur in oligotrophic water and increased nutrient and ammonia levels have been found to cause reduced larval survivorship (Basslim & Sammarco 2003). Culturing corals in indoor tanks with the use of artificial light is also an option in culturing projects, but would increase the cost and necessary resources needed for most local reef managers so was not used in this project.

2.15 Training local stakeholders and volunteers

Topics involving coral spawning were discussed the monthly SKT Marine Branch meetings with the community for the months leading up to spawning to increase awareness and encourage participation in the study project. Divers from the NHRCP and other dive centers (mostly dive instructors) were given three 60 minute lectures on coral identification and reproduction, coral spawning and culturing basics, and coral gamete collection procedures. During the nightly spawning timing observation period, divers were briefed on the procedure and practiced mock-spawning exercises in preparation for the rapid deployment of dive teams following the observation of corals preparing to spawn gametes.

Dive buddy teams working in 20 minute rotations maintained constant observation of the selected adult coral colonies while the other divers waited on the boat. Twenty minute intervals provided the regular communication and updates on the status of the corals being observed. When coral colonies were observed to ‘set’ (prepare for broadcast spawning) a visual (waving dive torch) and auditory signal (whistle) was used to signal the waiting dive volunteers on the boat.

2.16 Collecting coral gametes

Prior to the spawning observation and gamete collection dives, the three sites in Chalok Ban Kao were marked using color-coded nylon rope. The rope was run between coral colonies to facilitate easy navigation of the site and movement between the selected adult coral colonies by the volunteer divers. The center of each site was marked with a temporary buoy and colored strobe (purchased at a fishing store) to allow waiting divers to quickly and efficiently get to the spawning sites when signaled to do so. A mesh bag containing extra nets and collection jars was secured to the base of each temporary buoy.

Volunteer divers waiting on the dive vessel during spawning were assigned to one of the three collection locations, or coral sub-populations. Gamete collection procedure was based on a modified version of the methods found in Verimeij *et al.* 2006 and Verimeij *et al*. 2009. In addition to standard night diving equipment for SCUBA diving, each gamete collecting buddy team was equipped with a modified butterfly net. The nets are used to collect the buoyant coral egg/sperm bundles as they are released from the target colonies. The use of nets placed over corals prior to spawning as described by Verimeij *et al. (*2006) was experimented with in the first two years of the project, but was abandoned due to its adverse effect on coral health and the fact that netted corals are then removed from the natural reproductive population. Volunteers were instructed to collect no more than 50% of the total reproductive output from any single colony, and to collect bundles from several colonies within each distinct subpopulation in order to maximize the genetic diversity of the collected gametes.

Egg and sperm bundles collected in the butterfly nets were then released by a screw top at the top of the butterfly net into a clean, sealable jar marked with colored tape corresponding the collection location and species. This was done underwater to reduce the potential loss or breakage of the egg bundles. The jar was then transported quickly but carefully back to the dive vessel for fertilization.

2.17 Fertilization of coral gametes

Fertilization of the gametes was conducted in several buckets of clean sea water. Buckets were filled a few hours before spawning began to reduce the chances of the water containing unwanted non-coral organisms or sperm from spawning coral colonies. Once back to the dive vessel, the jars containing the coral larvae embryos collected by diving volunteers were fertilized according to the methods of Heyward (1999), modified to the theories of this study with the goal of maximizing the diversity of genetic mixing. Accordingly, a percentage of the gametes collected from each sub-population was mixed with those from another sub-population of the same species. This is done to reduce the amount of genetic bottle-necking in restoration by allowing fertilization between individual coral colonies that would normally be too distant to

interact reproductively. For the 2013 *Favidae* spawning, corals were collected from three sites (A, B, and C) which were separated by 70-120 meters. Fertilization took place in 4 buckets (A+B, A+C, C+B, A+B+C), with 33% of the gametes from each jar being poured into the various fertilization vessels.

The density of gametes in any single fertilization container was kept to about 80-90% coverage of the water surface (Edwards 2010) to maximize the amount of interaction between coral germ cells without compromising the water quality or concentration of dissolved gases. The gametes were gently agitated with an aquarium air pump and air stone placed at the bottom of the tanks to encourage mixing and provide oxygen to the metabolizing and developing eggs. Clumps or aggregations of eggs were gently separated using a Pasteur pipette or other small volume wash bottle. Agitation of the fertilization vessels may also help to increase larval yields through cleavage and the asexual cloning of coral embryos (Heyward & Negri 2012). Gametes were allowed to fertilize for 1-1.5 hours, at which time the fertilized cells were transferred to the culturing units.

2.18 Culture of coral embryos and larvae

Before transferring fertilized coral embryos to the culturing tanks, the airstones were shut off to allow the buoyant embryos to float to the water surface. Care was taken to avoid transferring excess sperm concentrated in the fertilization tanks to the culturing tanks as the breakdown of the sperm will reduce water quality. Embryos were carefully scooped from the surface of the fertilization buckets and poured into the culturing units which had previously been filled with clean sea water. Air stones and pipettes were used to break apart clumps of cells as in the fertilization procedure.

The density of embryos placed into any single culturing tanks was less than that of the fertilization vessels, around 30-40% of the total water surface. Water was slowly circulated through the flow-through system to maintain physical conditions and water quality at ambient levels. For this study, the water outflow from the tanks was designed to reduce the loss of larvae by placing the outflow tubes at the bottom of the tank, and allowing the water to flow out of a dispersed network of small holes to reduce the current at any single point (Figure 9, in Appendix F). Air flow (through a commercially available ‘air curtain’) and water circulation were kept constant 24 hours a day, and volunteers checked the system for problems or declines in water quality every 2-4 hours during the daytime.

During the first 24 hours, the breakdown products from unfertilized gametes and the metabolism of developing embryos will be high, and can quickly lead to poor water quality.

Water changes were performed every 6 hours for the first 36 hours post-fertilization, replacing 50% of the volume of the sump tank (about 15% of the total culturing unit volume). After the first 36 hours, the frequency of water changes was reduced to once per day, correlating with the daily high tide and avoiding the mid-day hours when the shallow seas are warmest. A digital thermometer was placed in each tank, as a study by Bassim & Sammarco (2003) have found temperature increases from 28°C to 30°C and 30°C to 32°C resulted in 50% and 70% greater larvae mortality rates (respectively). In the event that temperatures in the tanks increased, water flow to the tank would be raised. It was theorized that water temperature would greatly fluctuate in the tanks, and in extreme cases it was planned to use sealed bags of ice inside the tank to reduce temperature during peak sunlight hours. However the temperature buffering ability of the concrete proved sufficient to passively maintain a constant temperature at ambient levels (daily average of 28.8°C).

2.19 Settling larvae

The coral larvae in the culturing tanks were observed daily for color, size, and swimming behavior. Larvae are known to be settlement competent when they are observed to swim down to the bottom of the tanks and explore the substrate (Vermeij *et al*. 2009, Edwards 2010). After approximately 4 -8 days, the larvae reached a settlement competent stage and artificial substrates were introduced to the tanks along with necessary chemical triggers obtained from the natural reef.

For the second and third year of this project, artificial substrates were created using a modified version of the "Coral Peg" developed by Omori (2009) in which cement is mixed with beach sand and molded in small plastic candy tubs. A large plastic wall anchor was inserted into the base of each molded concrete during setting, which facilitated a cheap and standardized mechanism of transport and securing throughout the project. After curing, the artificial substrates were attached to small plastic trays and preconditioned on the reef at a depth of 5 meters for a period of 1-6 months to allow stabilization of pH and the growth of Crustose Coralline Algae (CCA) and biofilm. In 2013, the substrates were conditioned for over 1 year, as substrates used in the 2012 project and placed on mid-water coral nurseries, but not containing successful juvenile corals, were reused. Before being introduced to the tanks, the substrates were cleaned of any macroalgae, tunicates, sponges, or other fouling organisms. CCA however was not targeted for removal, but generally suffered high mortality rates during the cleaning process

Coral larvae naturally respond to environmental triggers on the reef including CCA, and bacterial biofilms to initiate settlement (Morse *et al*. 1996, Vermeij *et al.* 2009, Negri *et al.* 2001, Harrington *et al.* 2004). In order to imitate natural inducers for settlement, rubble covered in

various species of CCA was collected from the reef at the same depth as the parent populations for the larvae (3-5m). These pieces were then roughly ground in a bucket of sea water, which was then introduced to the individual tanks over a period of 2 days. After about 48 hours, depending on the coral species used, the majority of the larvae have settled, and little to no larvae were observed swimming in the water column.

2.20 Rearing settled coral larvae

After settlement of the larvae, the water and air flow to the culturing tanks was increased to maintain a high level of water quality and imitate the currents that would naturally be found on a reef. Settled coral larvae were maintained in ambient conditions, under natural light regimes, for between 1 week and 6 months. A few fragments of living corals were placed into the tanks to provide a source of zooplankton for the settled corals. Fresh sea water was introduced to the system daily, containing zooplankton as a potential food source for the settled corals, if filtered or artificial sea water is used, brine shrimp can also be introduced as food for the developing polyps.

In the rearing stage of this project, water changes of about 10-20% total system capacity were carried out daily. Water changes were conducted during the highest tide of the day, or in the early morning, to ensure low temperatures, nutrients, and dissolved solids in the fresh sea water. Following heavy rainstorms or during very low tides water changes were not performed for up to 1-3 days.

Risk management is an important aspect of any restoration project, especially those being undertaken by local reef managers. High mortality of coral larvae or juveniles can be experienced due to only small decreases in water quality or temperature. Based on the experiences in 2010-2012, risk mitigation guidelines were included for the final year of the project. This was generally accomplished by splitting half of the stock at every stage of the project. After fertilization, about half of the larvae can be poured back into the sea, artificially raising fertilization rates and allowing for cross breeding or hybridization without necessitating the culturing steps. After the larvae become settlement competent, half of the larvae can be released into the restoration zone using jars or the techniques described in Heyward *et al.* (2002). One week after settlement on artificial substrates in the culturing ponds, half of the settled coral larvae were moved to a floating mid-water nursery near the parent reef. Thus for the remainder of the project half of the settled coral were being reared *in situ*, and half *ex-situ*.

2.21 Tracking mortality and growth

Successful coral recruits from this project have been followed over the course of several years to track for mortality and growth. Recruits are regularly photographed for size analysis in CPCe or directly measured to track growth. As coral recruits mature in the marginalized areas or the mid-water nurseries, they will be monitored by measuring growth rates using a ruler and photographs *image*J software, monthly quadrant surveys, and monthly 100 meter transect lines on the adjacent reef. Data between the various control groups will be analyzed using Excel and ArcGIS, and additional data on naturally occurring coral recruits in the marginalized areas will be followed to find if there are any differences in fitness or growth between the naturally bred or the artificially selected/hybridized corals.

Chapter 3 – Results and Discussion

3.11 Assessing the impact of bleaching in 2010 and identifying resilient colonies

The historic November-December monsoons on Koh Tao brought low rain fall in late 2009. Subsequently, sea water temperatures (SSTs) exceeded 30°C on March 3rd, 2010, SSTs continued to rise, exceeding 31°C by April 15th, and 32.6°C on the 21st of May. The highest temperature recorded by the probes during the event was 33.4°C on May 20th (Chalok Ban Kao, depth 5 meters). From the 23rd of May to the 15th of June, temperatures remained between 31° and 32°C, and then increased above 32°C before gradually declining to 30.4°C by mid-July (See Figure 3 and Table 2 in Appendix B).. The highest average temperatures for the period were between the 4th and 24th of May (average 31.8°C), which also coincided with the highest bleaching rates observed on the island.

Reef monitoring surveys conducted through the locally designed Ecological Monitoring Program showed high variability in the degree of bleaching and subsequent mortality around the island and within individual bays. At the peak of the bleaching, around June 15th 2010, up to 96% of the corals surveyed were recorded as 'Partially Bleached' or 'Fully Bleached'. At the end of the event in September, data across all sites shows 39% coral mortality, with only 10% of corals bleached, leaving about 50% healthy. Most of the corals surveyed which suffered complete mortality by October 2010 were of *Acropoa* (86%), followed by *Poccilioporа* (61.5%) , *Fungia* (30%), and *Montipora* (25%) (n=434, sample population includes 5 sites from around the island, shown in Table 1 below). A map of bleaching was created using arcGIS, and was published in the paper by Hoeskema *et al.* (2013), found in Appendix A.

Table 1: Temporal Progression of Coral Mortality by Genera (2010)

	Coral Genera													
	Acropora	*Diploastrea heliopora*	*Echinopora*	*Favia*	*Fungia*	*Goniastrea*	*Goniopora*	*Leptoria*	*Montipora*	*Other*	*Pavona*	*Platygyra*	*Pocillopora*	*Porites*
July	0.0%	0.0%	0.0%	0.0%	2.5%	0.0%	7.7%	**	0.0%	0.0%	3.3%	0.0%	20.0%	0.0%
Aug.	64.1%	0.0%	28.6%	50.0%	10.5%	0.0%	**	0.0%	17.6%	8.8%	6.4%	12.5%	71.4%	8.7%
Sept.	58.1%	0.0%	**	13.3%	**	9.1%	**	0.0%	25.0%	20.0%	0.0%	16.7%	66.7%	4.3%
Oct.	86.7%	0.0%	**	**	30.0%	0.0%	0.0%	**	25.0%	54.5%	0.0%	**	61.5%	18.0%

Data collected through the locally designed Ecological Monitoring Program (Scott 2012) clearly showed variation in the degree of coral mortality observed around the island. Deeper

areas generally fared better, most likely due to lower temperatures and light levels or the prevalence of thermoclines. Areas dominated by *Acroporides* or *Poccilioporides* prior to the bleaching generally suffered higher rates of mortality than those dominated by other coral types (See Figure 4 in Appendix C). Chalok Ban Kao showed the highest rates of coral mortality for any of the survey areas, despite the fact that this reef suffered high mortality rates in the worldwide bleaching event of 1998. The loss of these structurally important corals most likely will have a negative effect on local fishery stocks and tourism revenue for many years following the event.

The shallow reef of Chalok Ban Kao (3-6m) was found to have the highest mortality rate around the island, and is shown in *Figure 1* below. Prior to 2010, the area experienced less than 1% coral bleaching or mortality based on 8 surveys along the permanent transect line from 2006-2010. Mass coral bleaching began in early April of 2010, peaking around June 2 with 87% of the corals coded as 'Partially Bleached' or 'Fully Bleached.' Mortality of corals due to the bleaching was first observed in the area in early June, rising to an average 64% of the corals being coded as 'Dead' between 4 surveys conducted in September and October 2010 (See Figure 4, Appendix C). One of the main reasons for the high rate of mortality in the area was the high abundance of *Acroporidae* and *Pocillioporidae* corals and the lack of circulation in the restricted, shallow bay.

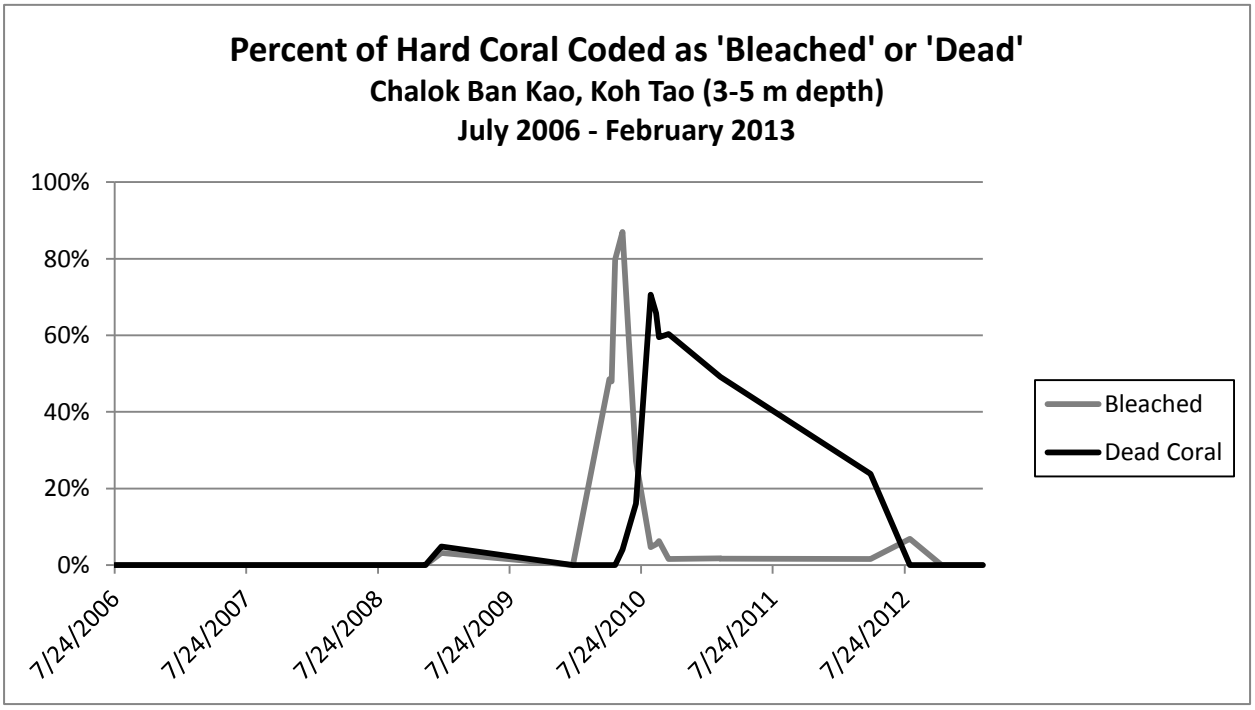

Figure 1 *– Graph of coral health in the shallow zone (3-5m) of Chalok Ban Kao from 2006-2013, showing the percentage or coral coded as either 'Bleached' or 'Dead'. The graph is based on 24 surveys conducted over the period in which both the permanent start and ending point were found when laying out the transect line.*

Coralivores predators after the bleaching event also led to further loss in coral coverage, primarily in the *Acroporidae* and *Fungidae* corals (Hoeskema *et al.* 2013). Due to the bay's high mortality rate and proximity to the culturing station, it was selected as the targeted collection and restoration site.

Two coral families were chosen for the project, *Acroporidae* and *Favidae*, based on the bleaching information and also published records of coral spawning timing already known for the area. *Acroporidae* was chosen as prior to the bleaching event this was the dominate form of coral observed in the area (70% of total coral colonies, n=143), and suffered the greatest mortality in 2010. It was assumed that any colonies surviving in this area would be robust and temperature tolerant, and possibly of higher genetic fitness than their dead counterparts. *Favidae* was the second family chosen as it is relatively abundant in the area (specifically of the genera *Goneastrea* and *Symphillia*), yet very few colonies suffered complete mortality in the bleaching event, making this coral potentially more useful in the long-term maintenance of coral coverage through local restoration efforts.

It should be noted however that this project did not complete any genetic sampling or molecular analysis, and there was no attempt to identify any more resilient genotypes within the targeted adult corals. This study, being focused on local reef managers, looked solely at the epigenetic results of a presumed higher tolerance to coral bleaching. Other factors may have also contributed to the survival of some colonies such as shading, pre-bleaching stress levels, depth, or post-bleaching predation by coralivorous *Drupella* snails. Identification of high genetic fitness corals is possible through observed epigenetic features such as fecundity, growth rates, lipid content, skeletal density, and other quantitative techniques. However, in reefs not directly following bleaching or other large scale disturbances identification of more resilient colonies will be much more difficult for local reef managers who generally do not have the resources or training for such analytical techniques.

3.12 Establishing schedules of coral spawning

For each family or genus of corals, isolated sub-populations were identified and mapped within Chalok Ban Kao. *In situ* histology and spawning timing observations were recorded for the duration of the study and compiled in excel to create a table of local spawning timings (see Table 1 on the following page). The timing of coral spawning fluctuated by 1 month between 2012 and 2013, generally occurring on the 4^{th} or 5^{th} day after the most recent full moon, within several hours after sunset. The earliest the corals were observed to spawn was *Symphillia* in 2013, at 7:03pm, the latest was Acropora in 2010, at 8:56pm.

Table 2: Spawning Timing observations on Koh Tao, Thailand

Year	Full moon (CET)	Full Moon (Bangkok Time)	Actual Spawning Time	Time after full moon	Hours after full moon
***Acroporidae* Spawning times**					
2010	2/28/10 17:37	2/28/10 23:37	3/4/10 20:56	1/3/00 21:19	93:19:00
2011	2/18/11 21:35	2/19/11 3:35	N/A	N/A	N/A
2012	3/8/12 10:39	3/8/12 16:39	3/11/12 19:59	1/3/00 3:20	75:20:00
2013	1/27/13 5:38	1/27/13 11:38	N/A	N/A	N/A
***Symphillia* Spawning Times**					
2010	3/30/10 4:25	3/30/10 10:25	4/4/10 19:28	5 D, 9 H, 3 M	129:03:00
2011	2/18/11 21:35	2/19/11 3:35	N/A	N/A	N/A
2012	4/6/12 21:48	4/7/12 3:48	4/11/12 18:59	4 D, 15 H, 11 M	111:11:00
2013	2/25/13 21:26	2/26/13 15:26	3/3/13 19:03	5 D, 3 H, 37M	123:37:00
***Goneastrea* Spawning Times**					
2010	3/30/10 4:25	3/30/10 10:25	4/4/10 20:18	5 D, 9 H, 53 M	129:53:00
2011	2/18/11 21:35	2/19/11 3:35	N/A	N/A	N/A
2012	4/6/12 21:48	4/7/12 3:48	4/11/12 19:49	4 D, 16 H, 1 M	112:01:00
2013	2/25/13 21:26	2/26/13 15:26	3/3/13 20:21	5 D, 4 H, 55M	124:55:00

Following the bleaching event of 2010, none of the targeted corals developed gametes or spawned during the normal season in 2011, consistent with observations from other regions following mass bleaching events (Ward *et al.* 2000, Baird *et al.* 2002). In the spawning season following normal growing seasons, *Acroporid* corals tended to spawn in March, except in 2013 when spawning was not observed, but assumed to have occurred in February. In the three years when spawning was observed, it tended to occur about 30 minutes to 1 hour following sunset, lasting approximately 35 minutes.

Acroporid corals tended to spawn one month prior to the *Faviidae*, which spawned in April in 2010 and 2012, and March in 2013. For the *Favidae* spawning nights, *Symphyllia* tended to spawn first, approximately 30 minutes to 1 hour after sunset, with *Goneastrea* being the second, about 1.5-2 hours after sunset. *Platygyra* and *Leptoria* were also observed to spawn in 2010 and 2012, approximately 2-2.5 hours after sunset on the same night as *Symphilia* and *Goneastrea*. The tables above were used to successfully predict the night of spawning in 2013, after eggs had already been observed through *in situ* histology by divers in February. The results of the spawning timing observations are planned to be published to contribute to available knowledge on spawning timings on Thailand's reefs (Scott and True, in prep.).

3.13 Developing a guide of reef restoration for increased genetic diversity for local reef managers.

The coral gamete collection and rearing projects of 2010 and 2012 provided as pilot projects for testing the utilization of local reef managers in genetically based coral restoration. Using non-professional teams and assistants proved difficult when using many of the techniques described in the literature (eg. Edwards 2010, Omori *et al.* 2010, or Heyward *et al*. 2002). But modifications were made throughout the program, using locally available and inexpensive materials to improve success amongst the non-professional teams.

In 2010, diver teams successfully collected gametes from both families of targeted corals alongside research students working under Dr. James True of PSU. Dr. True helped to train the volunteer divers and direct the project, using established techniques with some local modifications. Culturing of the larvae took place in small buckets and containers of sea water, which proved difficult to maintain a high degree of water quality and were subject to daily temperature fluctuations. Many of the developing larvae were also lost during the water changes, leaving very few larvae for settlement stage of the project.

The artificial substrates created for 2010 were constructed based on the ease of attaching to a floating mid-water nursery, but were placed individually into the culturing tanks for settlement. This meant that the substrates were handled or picked up multiple times during transplanting out to the nurseries, and were observed rolling around in the transport buckets on the way to the in sea location. Shortly after the substrates were moved to the nurseries, Koh Tao experienced island wide mass coral bleaching lasting from April to October, further reducing the success of the culturing project. Only 1 colony of *Goneastrea* survived on the artificial substrate after 1 year, but that colony has survived through the rest of the project. Although success was quite low, many lessons were learned on the difficulty for implementing the program amongst non-professionals in remote locations. Volunteers were asked throughout the project to give feedback and recommendations to aid future projects and the development of the manual created by this project.

For the project in 2012, three isolated populations of the each target coral family were identified and mapped in Chalok Ban Kao according to observations following bleaching. The three areas chosen included reproductive sized corals of the target families that would likely not be able to interact naturally during spawning events due to separation distances. Fertilization between these three distinct populations on the same reef is theorized to increase genetic diversity and reduce the effects of ‘genetic bottlenecking’ through intraspecific hybridization. Colonies which survived well in areas where most of the hard corals died were tagged for collection per the theory of increased genetic fitness. The targeted coral colonies were then

linked using nylon rope to facilitate the rapid movement of volunteer divers between coral colonies during spawning.

Volunteers were trained through a series of lectures and practical demonstrations on the collection and fertilization procedure, and instructed to collect from as many of the different colonies in their assigned population as possible. Although fecundity of the corals in 2012 was lower than in 2010, volunteers did successfully manage to collect gametes of both the *Goneastrea* and *Platygyra* genera of *Faviidae* corals. Teams of volunteer divers and conservation students took part in four spawning events over the period, the teams were demographically diverse, with ages ranging from 10 years old to 65 years old. Only one of the participants in all of the years held a degree in science at the Master's level or higher. Yet, using the techniques developed through the course of this study increased success and reduced costs and manpower with each successive spawning event.

Gametes were fertilized according to the theories of this project, with mixing between all of the sites (Sub-populations A, B, & C) to create intraspecific hybrids. Samples were mixed to maximize the degree of genetic variation in the produced larvae, decreasing founder effects and genetic bottlenecking. A high volume of successfully fertilized embryos was created, although no quantitative data was taken on the percent fertilization success. Future studies would also benefit to utilize a control population of embryos created by only local mixing as would occur naturally (A-A, B-B, etc). After fertilization, approximately 30% of the fertilized cells were poured back to the reef, to facilitate the selective breeding program and the higher fertilization success achievable through the culturing techniques, yet allow the larvae to distribute and settle according to their natural regimes.

The remaining larvae were cultured in an inexpensive flow through system constructed using clear plastic tubs modified with stand-pipes, a filter, and an aquarium pump. The system provided more continuous water flow than previous years leading to higher water quality in the containers and less temperature fluctuation between day and night. However, volunteers were still required to carry water from the sea to the culturing units in buckets, making water changes labor intensive and thus liable to be missed or underperformed.

The artificial substrates used in 2012 were designed using a modified version of the technique established by Omori *et al.* (2009) and were preconditioned in the sea for a period of 3 months prior to use. The substrates were secured to plastic bases, so that volunteers would never need to handle them directly, and there would be no chance of the pieces rolling around during transport to the restoration site.

After 4 weeks of rearing in the tanks, a seasonal lack of volunteers meant that maintaining the culturing tanks would be difficult, and it was decided that moving all of the artificial substrates containing the settled larvae to the reef would be the best form of risk mitigation. Unfortunately, the author was unable to attend the transport and relocation to the mid-water nurseries, and volunteer error led to many of the substrates ending up in the sand below the nursery. Thirty seven months post-settlement, 1 individual colony measuring 89.1 cm^2 of *Goneastrea* had survived from the 2010 project on a mid-water coral nursery in Ao Leuk Bay. Seventeen months past settlement, the 6 remaining colonies from the 2012 project placed in Chalok Ban Kao Bay ranged in size from 0.8–3.1 cm^2. The number or size of survivors from the project for 2013 cannot be assessed until early 2014 when they will be large enough to be visible. (see photos in Figure 10, Appendix G).

The manual on a *Genetic Approach to Coral Restoration* (Scott, in press) was developed before the spawning season of 2013. The methods contained are a culmination of published knowledge and experience learned locally through 2010-2012. Over the course of the 2013 project, the techniques described were implemented and tested. Diving teams proved to be more effective and there was little to no error in the collection and fertilization procedures of the project.

Coral culturing was carried out in a culturing unit constructed specifically for this project using rendered cement. The culturing unit was preconditioned for 6 months prior to spawning, while simultaneously functioning as a nursery for sea turtles. The unit was constructed entirely out of locally available materials, and cost less than 100,000 Thai Baht to install and run for 1 year. Water quality, light, and temperature were continuously maintained using the unit, and water changes were facilitated by a submersible pump placed in the sea, 20 meters from the beach. The designs of the unit are included in the manual, along with other recommendations and tips for success that are easy to follow by non-professionals and local reef managers. The manual is set to be printed and distributed to local reef managers prior to the spawning event of 2014.

As in other stages of the project, to further mitigate risk, the stock of coral larvae and settled colonies has been divided between both *in situ* and *ex-situ* rearing. It is not known at the time of writing how many colonies have survived, but the project will be monitored over the next several years to track success and identify further problems or areas for improvement.

Currently the program is only being undertaken in the location of Chalok Ban Kao Bay, through increased training it may be possible to have similar projects underway in all of the marginalized areas around the island. Due to the high density of various marine conservation programs and courses on the island (currently 6 dive centers), Koh Tao is an ideal location to

continue this study and monitor the effectiveness of restoration techniques on a larger temporal or spatial scale that currently exists in the published literature. Due to the slow growth of corals, it may take more than a decade or more to fully quantify the benefits of such programs.

. Through the knowledge and methods gained through this study, a practical guide to integrating theories of increasing the genetic variability of feedstocks and hybridizing corals is in preparation. The guide is two part; first, an argument for genetic based management systems integrating case studies from projects conducted using coral ramets, and secondly an easy to follow procedure for carrying out the selective coral breeding and culturing project using volunteer teams and locally available materials. Such guidelines are strongly needed to preserve or restore the resilience of coral reefs in the face of a rising consortium of localized and global threats associated with human population growth and climate change.

Chapter 4 – Summary and Conclusions

Coral reefs are one of the planets most valuable and most threatened ecosystems. Those closest to the reefs (local managers) have the most stake in the survival of reefs, and play one of the most vital roles in their protection and restoration. To date, many coral management initiatives have proven unsuccessful due to overlooking of the importance of coral restoration, or by restoration efforts that are mis-guided or focused solely on the short-term increase in coral abundance through asexual propagation. As the effects of human activities and climate change become more pronounced, there will be an increased need for a shift in reef management towards long-term restoration and maintenance of reef resilience. In order to be effective, coral restoration programs must make a transition from the traditional methods of focusing on increasing coral abundance on reefs, to using an approach which factors in the genetic diversity of corals. The observed reproductive failure of corals on the island in 2011 following the mass bleaching event is a significant indicator of the need for such programs to assist in the recovery of reefs following large scale disturbances.

Data collected through the locally designed Ecological Monitoring Program (Scott 2012) clearly showed variation in the degree of coral mortality observed around the island, implying both variations in the degree of stress and resilience in the various reef locations. The data collected through this program not only assisted this project, but has also played a major role in the identification of 'No Take' or 'Restoration' zones during the design and implementation of an Island Marine Zoning and Regulation project undertaken by the Prince of Songkla University, DMCR, SKT Group, and Local Government in 2012 (Platong *et al.* 2012). The data is currently being used to assess the change in reef health around the island and also monitor the effectiveness of local restoration or management efforts.

Identification of resilient coral colonies to be used in the type of selective breeding program proposed by this paper was facilitated by the bleaching event of 2010. It was possible to select an area experiencing the highest rates of mortality through ongoing island wide monitoring as the target site for restoration. More detailed surveys identified coral colonies surviving well amongst the mostly dead reef, which were presumed to be more genetically fit than their deceased counterparts. It is expected, but not proven, that the embryos cultured from these parent colonies may be of a higher fitness and more genetically diverse due to the procedures developed in this project. More long-term studies are needed to confirm or refute this hypothesis.

Taking into account the effectiveness of selective breeding in both plants and animals, the studies on coral hybridization undertaken By Willis *et al.*(1997), or the recommendations by Baums (2008), programs which seek to increase genetic variability over short-term changes in

abundance should assist in the adaptation of reefs and make coral restoration and protection efforts more effective in the future.

In a situation where a mass disturbance such as coral bleaching has not recently occurred in a region as it had in this project, long-term monitoring of tagged coral colonies would be necessary. Individual colonies and populations of corals should be observed regularly and data recorded on growth rates, productivity, health, and fecundity. As this may be outside the ability and scope of many local reef managers, it may be advisable to focus instead on maximizing genetic variability by collecting from a healthy population of parent colonies and cross-fertilizing gametes between other reproductively isolated populations.

Encouraging hybridization between species spawning on the same evening may also assist in increasing the amount of genetic material available and allow the transfer of stress tolerant genes between populations or species (Van Oppen *et al*. 2003, Willis *et al.* 2006). Regardless of the exact techniques or procedures used, restoration through the creation of genetically diverse feedstock's of corals should be promoted over the current mainstream techniques of asexual propagation and transplantation. The increased success in coral reproduction and the creation of restored reefs using genetically distinct corals may greatly increase the long-term success of coral restoration projects in areas where other protection and mitigation efforts are also being implemented.

Over the course of this study, it was possible to undertake the coral gamete collection and larvae culturing program alongside local reef managers in both 2012 and 2013. Through the training given to the divers at the NHRCP, local managers from two other local marine conservation groups (*Eco Koh Tao* and *Koh Exist*) were also trained and participated in the project. The local community has been made aware of the project through a video and posters shown at a yearly environmental festival, or in more detail at monthly community meetings. In 2013, the project was also highlighted in an article and series of photographs in an international online magazine (Tyrell 2013

As in the case of this project, encouraging public participation and integrating these types of programs with courses on marine conservation allows such projects to be primarily self-funded, or may even provide new industries to preserve the livelihoods of local residents (Scott and Phillips 2010). The culturing tanks, boat fees, SCUBA equipment, and air tanks used over the course of this project were all paid for through funding generated by the sale of marine conservation courses at the NHRCP. The promotion of similar free market-based conservation and reef management models may help to drive environmental protection and decrease the high cost of restoration projects.

The spawning timings on Koh Tao fluctuated between months year to year, but were found to be consistent in timings after the full moon and sunset, leading to an accurate prediction of the spawning time in 2013, following the observation of pigmented gametes inside adult colonies in February. Still, only 5 genera of corals from 2 families have been observed spawning, and only over 3 years time. More information and studies will be needed over the next years to establish more accurate timing calendars and cover a wider range of coral families or genera. Further studies will also be needed to identify the levels of coral recruitment between various sites around the island to focus restoration activities in the areas where larval supplies or survival is low.

The program will continue on the island using the manual for coral restoration developed by this project in the coming years, and further add to the knowledge on spawning timings and the effectiveness of the techniques. Although training local reef managers and volunteers in the techniques proved successful, the full success of the techniques in coral restoration may not be realized for several years or decades, and further work is needed to evaluate the benefits of such programs over other coral restoration techniques. It is hoped that this project will provide multiple environmental and social benefits to both the study sites on the island of Koh Tao and to local or global reef managers. Furthermore, if the selective breeding program yields the expected results, than it may help to direct reef management towards more sustainable and successful restoration programs.

Although the techniques for coral gamete collection and culturing have been established and described for over a decade (Peterson & Tollrian 2001, Heyward *et al.* 2002, Edwards 2010, Omori *et al*. 2010), few projects are in place to use the techniques for active restoration. Little to none of the knowledge on coral larvae culturing is available to non-scientists, and the techniques utilized are generally expensive and require extensive training and experience. Furthermore, the timings of coral spawning are not available in most areas (Guest 2009, Kongjandtre *et al.* 2010), and little data is available on the sources or sinks of coral larvae between connected reef areas (Guest *et. al.* 2008).

To date, there are few practical guides for reef managers explain the coral larvae capture and culturing programs, and no guidelines have ever been written for the practical application of increasing genetic variability or assisting the adaptation of corals in restoration. The product of this project is a practical guide to integrating theories of increasing the genetic variability of feedstocks and hybridizing corals through the knowledge and methods gained through this study.. Such guidelines are strongly needed to preserve or restore the resilience of coral reefs in the face of a rising consortium of localized and global threats associated with human population growth and climate change. Although this report may help to create opportunities for reef

managers to practically address coral reef resilience and adaptation, it will take the continued work of a dispersed system of professional researchers and local reef managers to develop these techniques further. If current rates of reef decline continue the coral environments and their associated economies will be lost within the next century, but with a shifted focus and more scientifically informed techniques, coral reef management in the 21st century could be made more effective than has been shown over the last few decades. If widespread participation can be achieved, some vital marine resources may be sustained in the face of chronic stresses, development and climate change.

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Appendix A

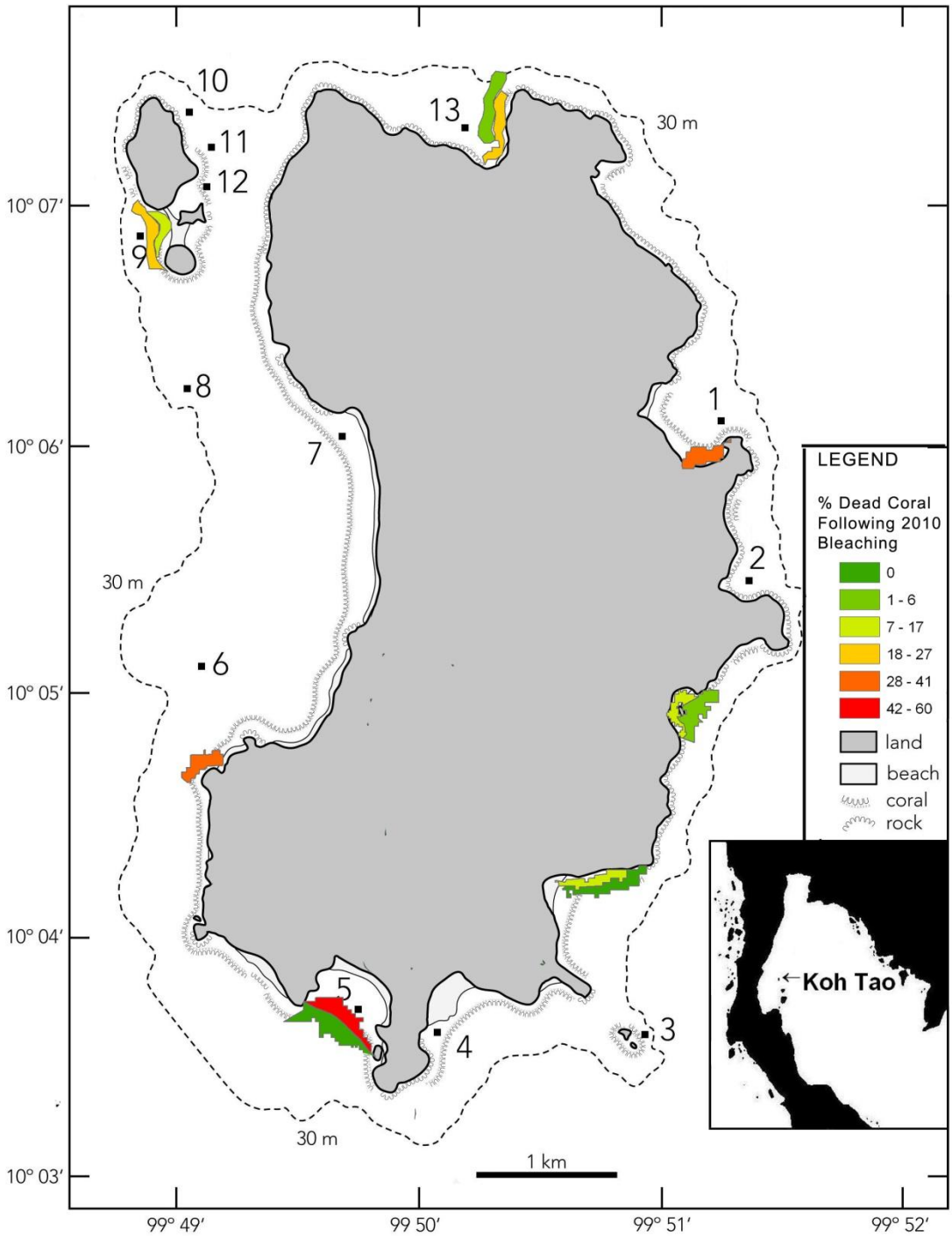


Figure 2 *- Map of Koh Tao showing coral morality following the mass bleaching event of 2010 for all areas with permanent transect lines under the locally designed Ecological Monitoring Program. From Hoeskema et al. 2013.*

Appendix B

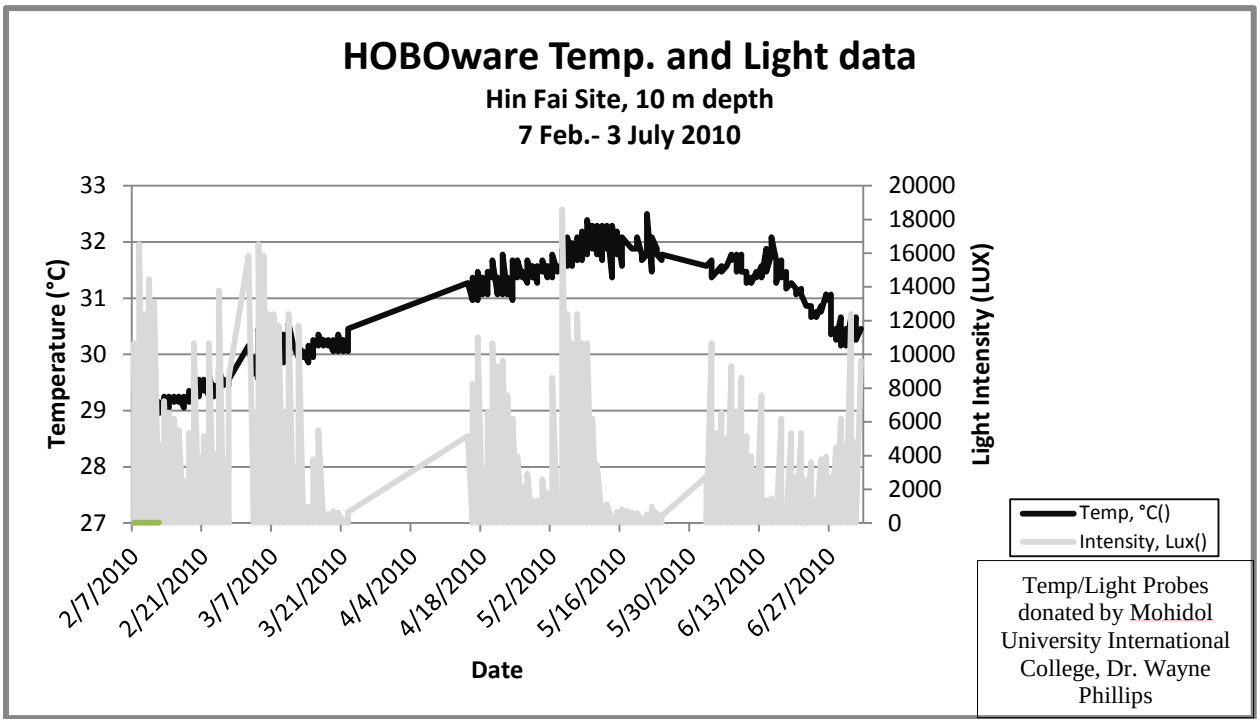


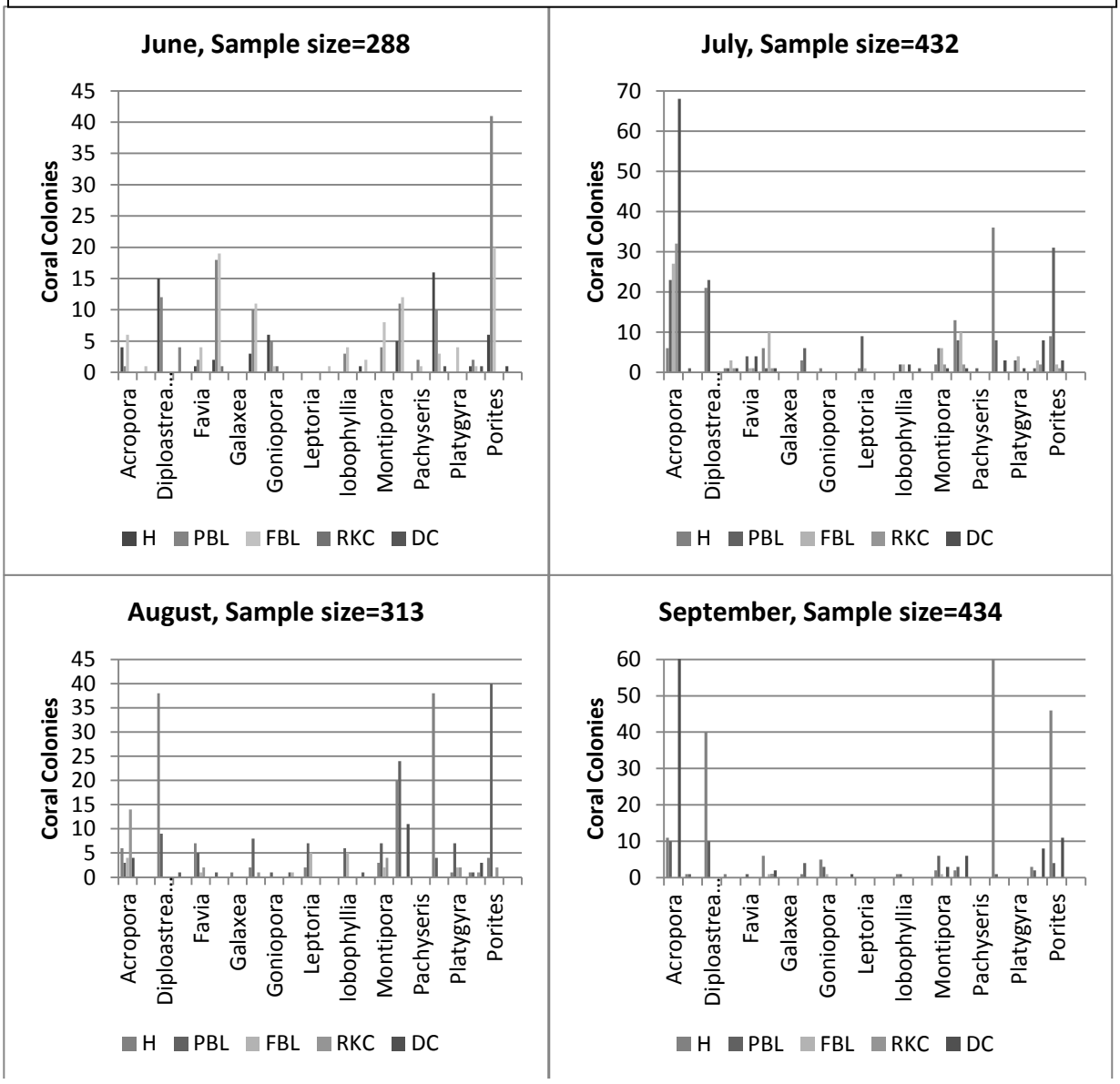
Figure 3 *- Sea water temperature and light intensity levels at the site of Hin Fai (10 meters depth). Temperatures exceeded 30.5°C on March 10th, with the first bleaching corals observed around April 9th. Sea Water temperature peaked at 32.5°C on the 21st of May, 2010.*

Table 3 *– Summary Statistics for sea water temperature at Hin Fai, Koh Tao (10 m. depth)*

Statistics for Series: Temp, °C								
• First Sample Time:	2/6/10	3/2/2010	4/15/2010	5/4/2010	6/2/2010	6/9/2010	6/17/2010	6/27/2010
GMT+07:00	3:34 PM	12:18	13:33	13:17	2:22	1:14	1:57	4:48
• Last Sample Time:	2/26/10	3/22/2010	5/4/2010	5/24/2010	6/9/2010	6/17/2010	6/27/2010	7/17/2010
GMT+07:00	6:23 PM	15:09	13:15	16:08	1:12	1:55	4:47	7:39
• Samples:	28,970	28,972	27,343	28,972	10,011	11,562	14,571	28,972
• Max:	29.752	30.659	31.983	**32.6**	31.88	32.086	31.983	31.37
• Min:	28.754	29.352	30.154	30.457	**31.268**	31.064	30.457	30.457
• Avg:	29.240	30.064	31.311	**31.836**	31.566	31.446	31.01	30.694
• Std Dev (σ):	0.192	0.188	0.28	0.275	0.13	0.186	0.298	0.143

Appendix C

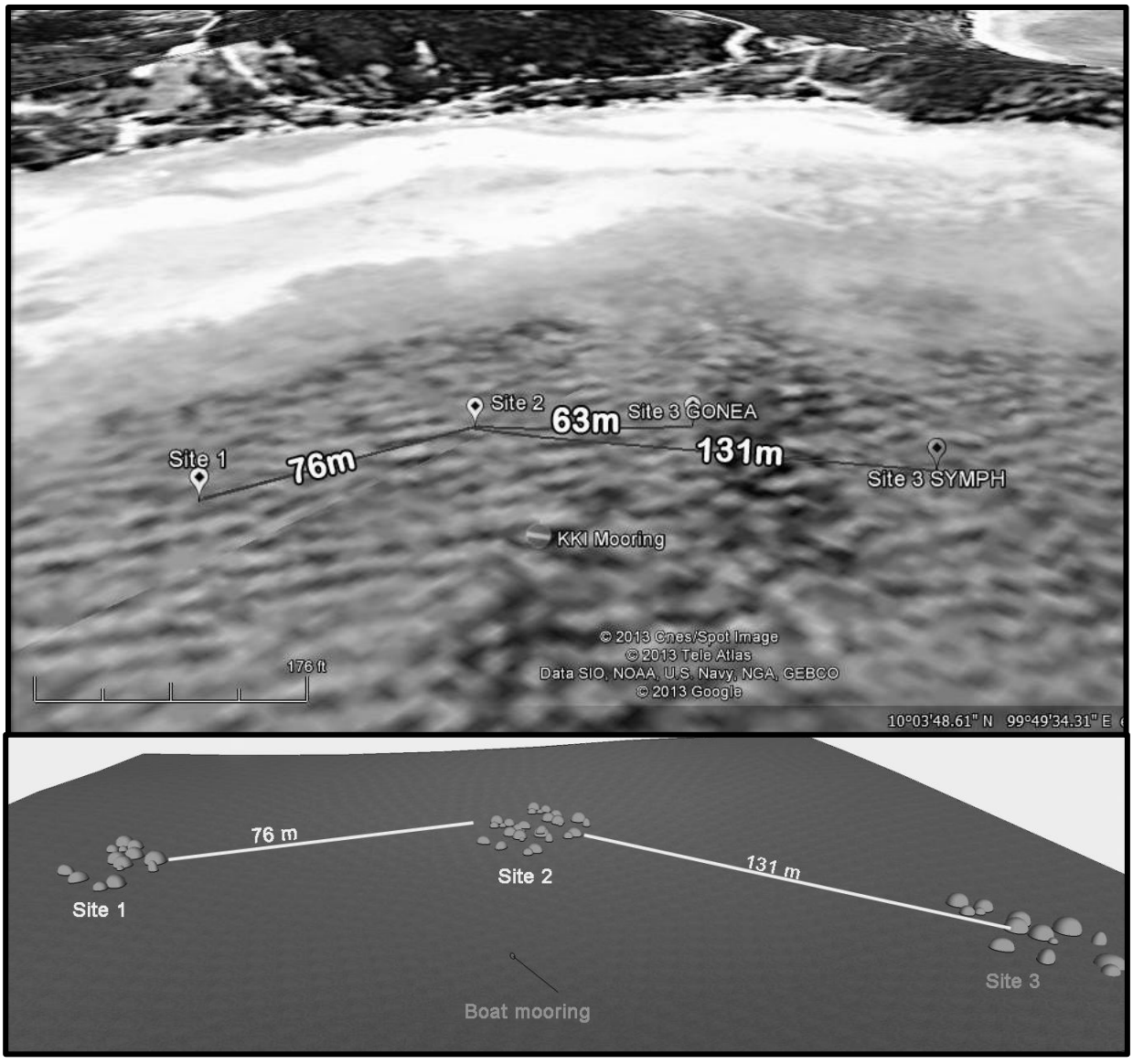
Figure 4 – Coral Bleaching by Genera. June-September 2010. Koh Tao, Thailand. All site surveyed.



Number of coral colonies marked as Healthy (H), partially Bleached (PBL), fully Bleached (FBL), Recently Killed (RKC), and Dead (DC). Data is summarized for all sites surveyed over the month, sample sizes ranging from 288 to 434 colonies as assessed along the permanent transect lines of the local Ecological Monitoring Program. The graphs show both the degree of bleaching and the impact of mortality on various genera of coral. Corals suffering the highest and lowest rates of mortality were both focused on during restoration efforts to improve the rebound and resilience of Koh Tao's reefs.

Appendix D

Figure 5 - *Map of coral gamete collection sites in 2013 in Chalok Ban Kao, Koh Tao. The first map shows the locations of the three breeding populations for both Symphillia and Goneastrea. The second conceptual drawing (below) shows the locations of the breeding populatiosn on the sea floor, between the three sites is a dense fringing reef dominated by Porites and Pavona corals. The populations are separated by at least 60 meters, and are expected to have little to no mixing or cross-fertilization in natural spawning events.*



Appendix E

Figure 6 - *Simplified drawing of the 2012 Flow through culturing system (piping and pump not shown)*

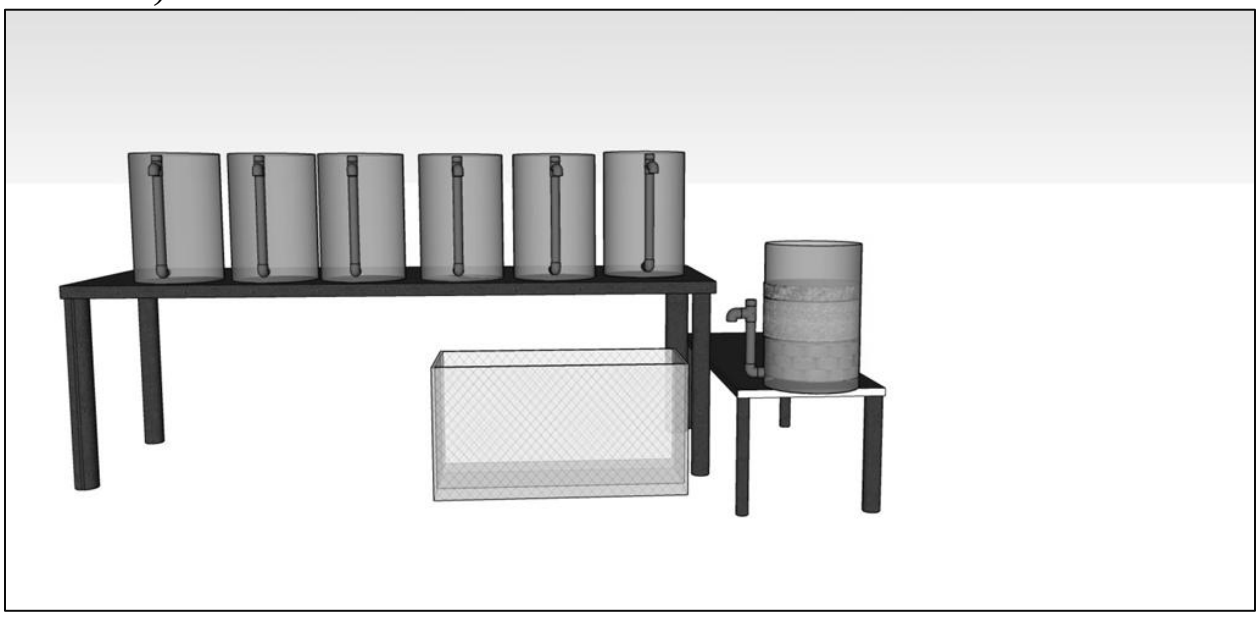
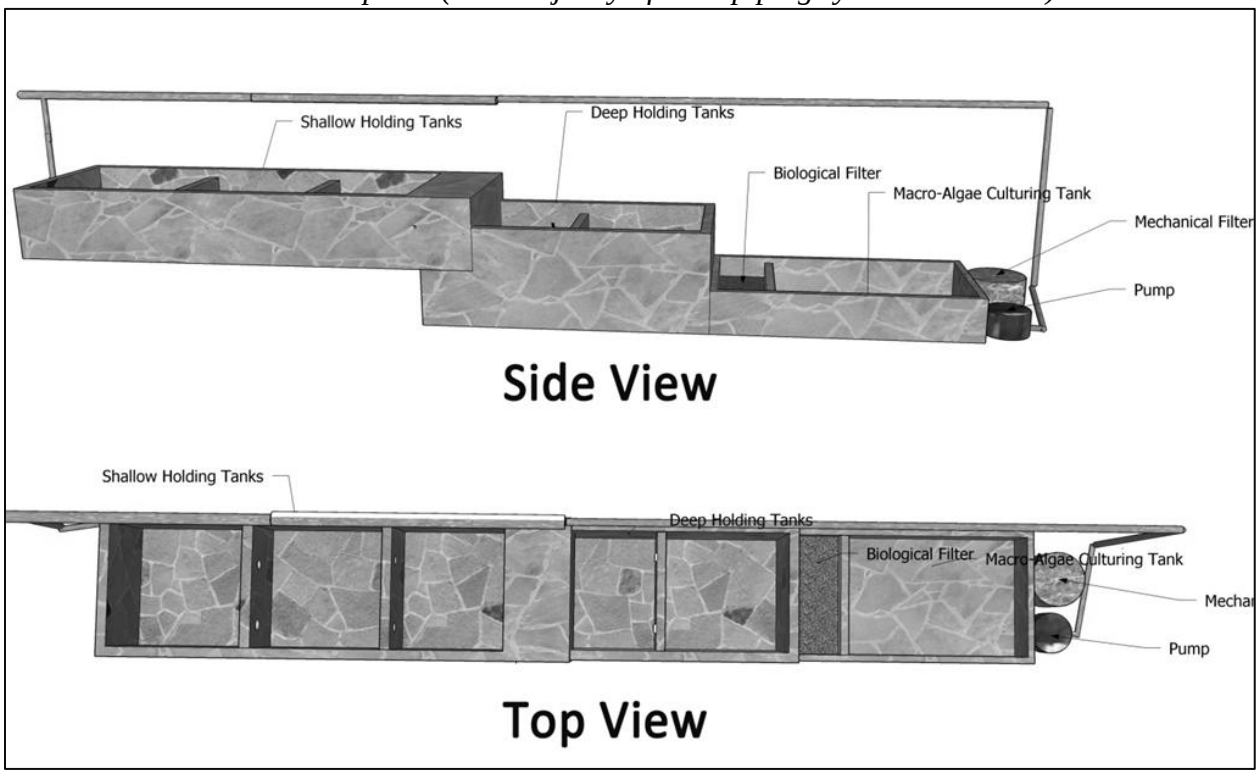


Figure 7 - *Design of the 2013 flow through tanks utilizing the standpipe design but with rendered concrete pools (note majority of PVC piping system not shown).*



Appendix F

Figure 8 - *Conceptual design of a single culturing tank, made out of locally available materials and utilizing the stand-pipe design to maintain a constant water surface height without losing the buoyant coral eggs/larvae.*

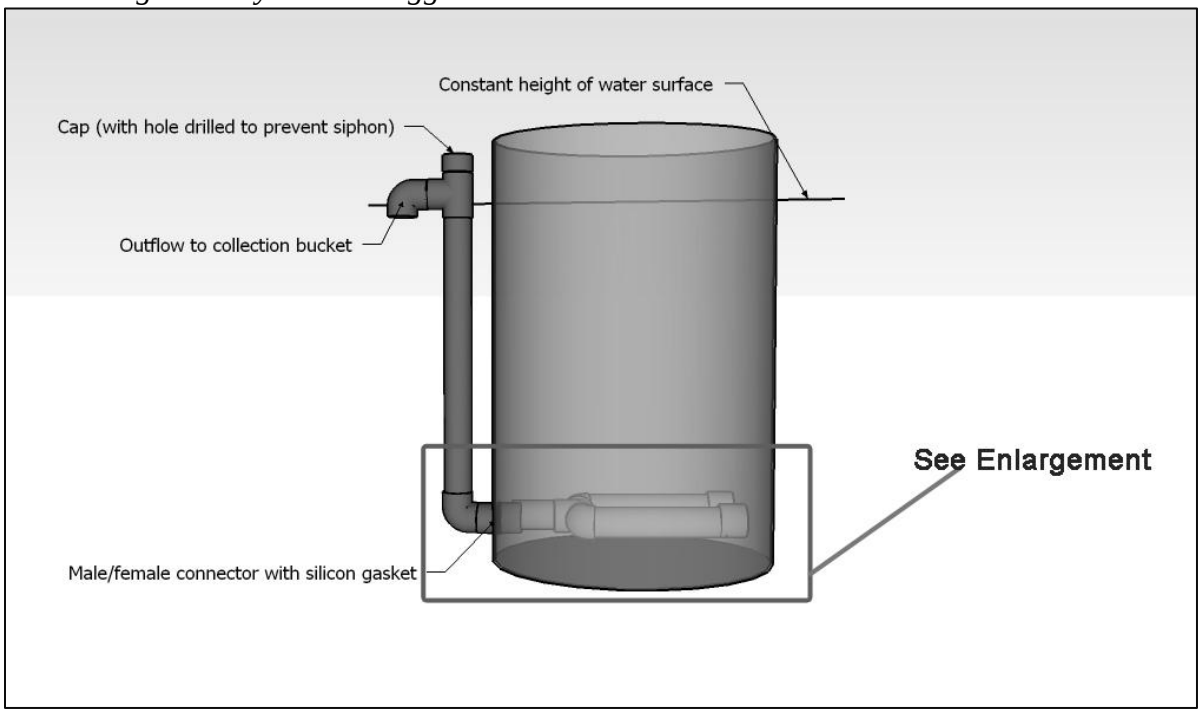
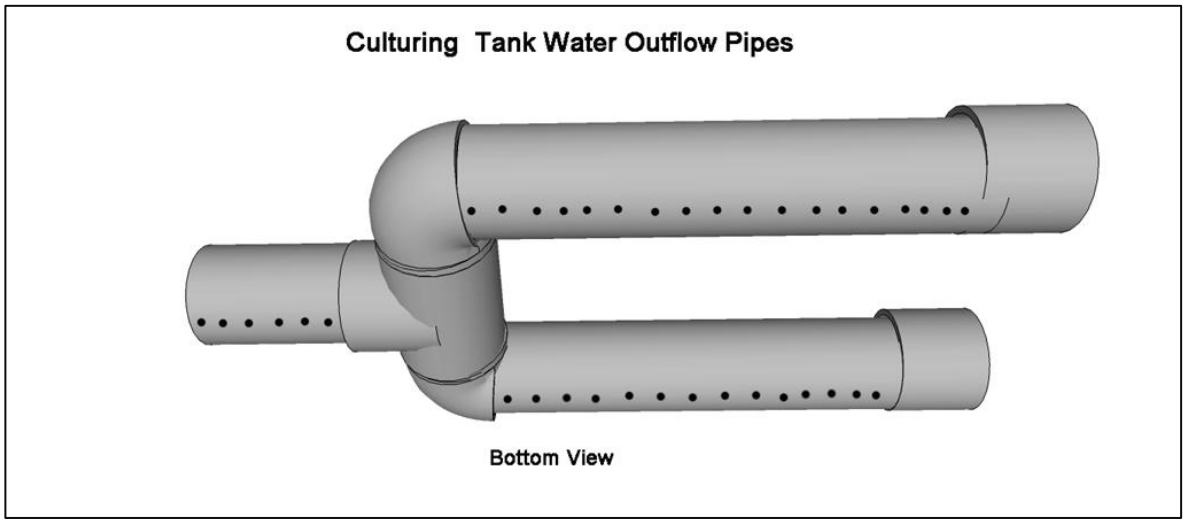
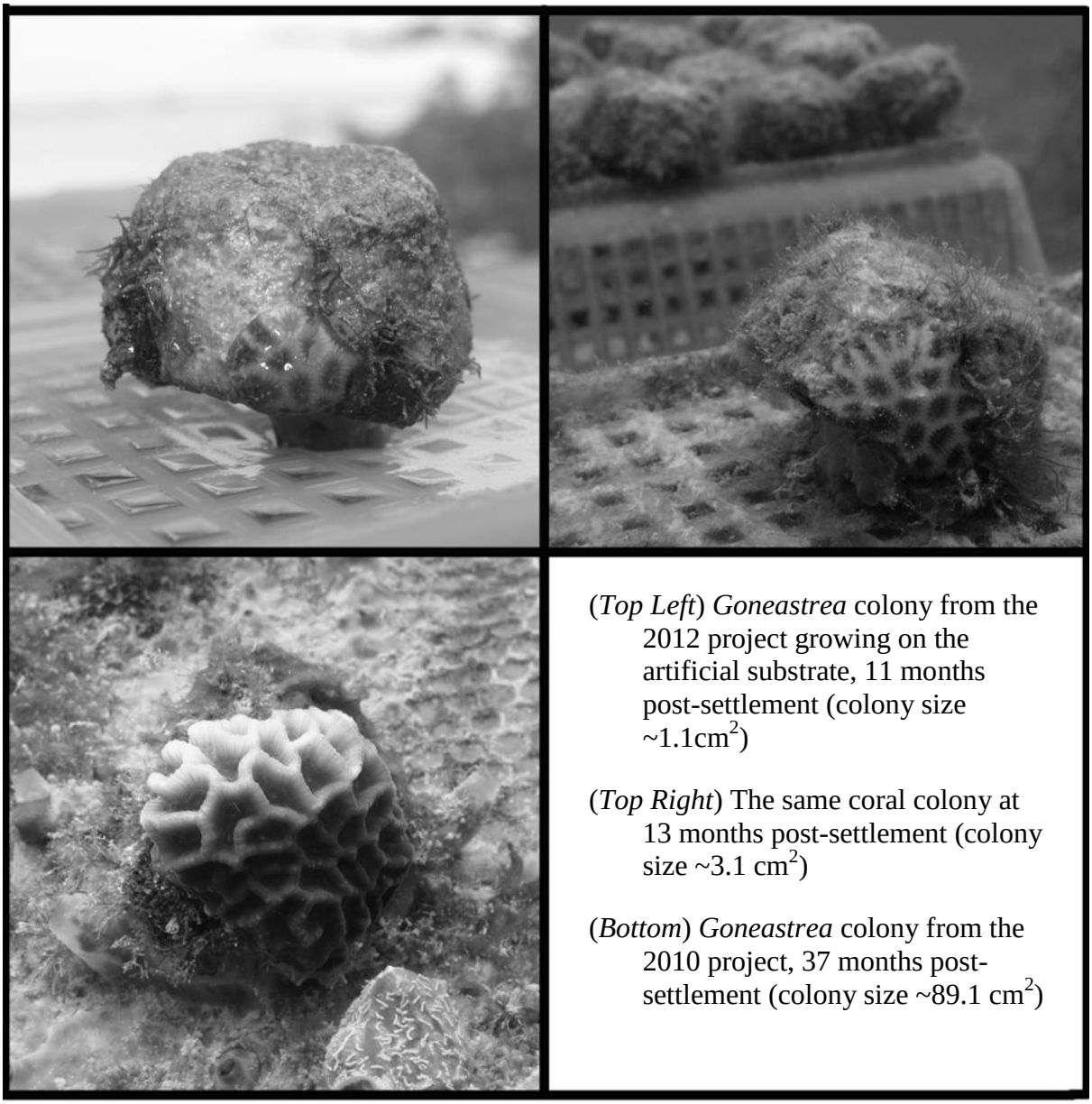


Figure 9 - *Enlargement showing the water outflow filter in the tank. The filter is designed to reduce the loss of coral larvae by slowly removing water from a diffused area and reducing suction at any given location.*



Appendix G

Figure 10 - *Photographs of cultured coral colonies from the project on Koh Tao, Thailand.*



(*Top Left*) *Goneastrea* colony from the 2012 project growing on the artificial substrate, 11 months post-settlement (colony size ~1.1cm^2)

(*Top Right*) The same coral colony at 13 months post-settlement (colony size ~3.1 cm^2)

(*Bottom*) *Goneastrea* colony from the 2010 project, 37 months post-settlement (colony size ~89.1 cm^2)

Vitae

Name: Mr. Chad Michael Scott

Student ID: 5310220129

Educational Attainment

University of Colorado 2003-2007
Boulder, CO 80309
Bachelor of Science in Environmental Studies, *Cum Laude*
Minor in Geological Sciences

Scholarship Awards during Enrolment

Work – Position and Address

New Heaven Reef Conservation Program, *Program Director*
48 Moo 3
Chalok Ban Kao, Koh Tao
Suratthani, 84360

Save Koh Tao Mairne Branch, *Project Coordinator*
Chalok Ban Kao, Koh Tao
Suratthani, 84360

List of Publications and Proceedings

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